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THE BOTANICAL REVIEW

VOL. VI

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No. 1

SEXUALITY, DEVELOPMENTAL CYCLE AND PHYLOGENY OF YEASTS¹

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OLDER NOTIONS

After Pasteur had demonstrated that alcoholic fermentation results from the activity of yeasts, these microorganisms, previously regarded as lifeless by many scientists, attracted the attention of botanists and became the subjects of morphological and systematic investigations. Thenceforth, the question was open: To which place among the fungi should yeasts be ascribed? The question was difficult to answer, for many fungi produce yeast-like cells (conidia) at certain phases of their development, cells which do not differ in appearance from true yeasts, but which may, after protracted clonal multiplication through repeated budding, revert to mycelial growth. These facts explain why Brefeld considered yeasts, not as fungi in themselves, but merely as phases in the development of higher fungi. Pasteur, haunted by the problem concerning the source of yeasts in grape juice, for a while concurred with Brefeld's opinion, and the idea ever since has been sporadically upheld.

Other botanists, on the contrary, among whom were Rees and de Bary, emphasized that yeasts form spores within their cells, which the yeast-like cells of higher fungi do not do. They rightly regarded these spore-containing cells as asci enclosing ascospores, and the yeasts, therefore, as belonging to the lower Ascomycetes, next to the Exoascales, which at that time were the only fungi with which yeasts could be compared. The Exoascales differed from yeasts only in producing a mycelium on which the asci were developed, but their ascospores, formed within these asci, budded into yeast-like cells before forming a new mycelium. Later, the Danish mycologist, Em. Chr. Hansen, through his precise and lifetime

¹ Article translated from the original French by J. Dufrenoy, translation re-written by the editor, and revised rendition approved by the author.

researches on the morphology and systematics of yeasts, reached the same conclusion.

For a while the idea appeared to obtain support from cytological researches by Janssens and Leblanc who observed that yeast cells about to produce spores show two nuclei which fuse prior to the nuclear divisions resulting in ascospore formation. This "caryogamy" was similar to that which Dangeard had described as a sexual process in the ascus of the *Exoascales*. However, caryogamy in the yeasts was still doubtful. At that time the nucleus in yeasts was a subject of discussion; scientists could not agree as to what was termed "nucleus," and many believed that the yeasts, like bacteria, had nothing more than diffuse or, at most, poorly organised nuclear material.

THE SPORIFEROUS SAC OF YEASTS CONSIDERED AS AN ASCUS

As early as 1901 (12) the present author demonstrated that yeasts have typical nuclei which do not differ from nuclei of other fungi. It subsequently became possible to study the cytological phenomena accompanying formation of the sporiferous sac and to demonstrate that the sac shows all the characteristics of an ascus. The nucleus undergoes two successive mitoses, sometimes three, and ascospores are formed when small parts of the cytoplasm condense around the nuclei originating from these mitoses. Most of the cytoplasm, full of reserve material, makes up the epiplasm out of which the ascospores, at first quite small, grow and enlarge, ultimately filling in most of the space within the ascus. There are usually four or eight ascospores within each ascus, according to the type, but the number may vary from one to four when one or more nuclei degenerate after having originated through the mitoses.

CONJUGATION PRIOR TO FORMATION OF THE ASCUS

The fact that we, too, had observed yeasts to conjugate did not permit us, however, to uphold the notions of caryogamy as reported by Janssens. It enabled us, however, to show for the first time (13, 14, 15) that isogamic conjugation occurs before ascus formation in the three then known species of the genus *Schizosaccharomyces* (*Sch. octosporus*, *Sch. Pombe*, *Sch. mellacei*). These three are peculiar yeasts from hot countries, differing from all others in multiplying not by budding but by transverse partitioning. In *Sch. octosporus* conjugation results from two vegetative cells assuming

the parts of gametes and fusing by means of a connecting canal, thereby forming a rather large oval zygote. A depression may show in the middle where the two gametes have come into contact. Two or three successive mitoses then resolve the zygote into a four- or eight-spored ascus. In the two other species of *Schizosaccharomyces* the gametes never fuse completely, and the resulting zygote retains two bulges, one at each end of the connecting canal, in the middle of which the two nuclei fuse into a diploid nucleus. The first mitosis yields two nuclei which migrate, one into each bulge, there to divide again and thus to differentiate the zygote into a four-spored ascus with two ascospores in each bulge. In the same year Barker (2) found a similar process in a budding yeast isolated from gingerbeer which he ascribed to the new genus *Zygosaccharomyces*, and which is now known as *Zyg. Barkeri*. It conjugates the same way as *Sch. Pombe* or *Sch. mellacei*, both zygote and ascus retaining the characteristic bulge at each end of the fusion canal. The ascospores, four as a rule, are formed in the bulges. Since then, many species of *Zygosaccharomyces* have been discovered by various authors and by ourselves.

Mlle. Manuel described isogamic conjugation in *Nematospora Coryli*, a parasite on hazel, which shows many peculiarities. The big ascus contains eight needle-shaped ascospores, each bearing a lateral flagellum, and results from complete fusion of two gametes, as in *Sch. octosporus*, into a large elongated ascus within which it is easy to watch the mitoses. Such conjugation may be considered to prove that Chatton was correct in reporting the same process, without, however, bringing forth proof for it in a similar yeast, *Coccidiascus Legeri*, a parasite of *Drosophila funebris*.

Our later researches (9, 10, 11) brought forth the existence of heterogamic conjugation in a new yeast, *Zygosaccharomyces Chevalieri*, later known as *Zygopichia Chevalieri* Klöcher; in all species of the genus *Debaryomyces* (24); and in several species of *Zygosaccharomyces* (e.g., *Zyg. Nadsoni*) (26). The heterogamic conjugation takes place between two cells of totally different sizes. One is a big adult cell assuming the part of a female gamete, the other is a young and not fully developed bud acting as a male gamete. Both unite through a canal, the male gamete discharging all its contents into the female which then develops into an ascus. All intermediate stages between iso- and heterogamy may be observed. Later, Nadson and Konokotine (35) discovered other yeasts, ascribed to the

new genus *Nadsonia*, which showed the same heterogamic fusion, except that the female gamete, having received the contents of the male, buds off its whole contents. The two nuclei fuse within the bud-cell which then differentiates into a one- to four-spored ascus.

Conjugation before ascus formation, therefore, is widespread among yeasts, occurring in the genera *Zygosaccharomyces*, *Zygo-pichia*, *Debaryomyces*, *Nadsonia*, *Nematospora* and probably in *Coccidiascus*. One of the main features of this conjugation which we emphasized at the very beginning of our researches and again later (17), is that it may operate between cells of a clon originating from a single cell, even between the two daughter cells from a mother cell. In *Schizosaccharomyces*, for instance, we see a cell dividing by transverse division into two daughter cells which develop into gametes and conjugate into a zygote. Again, among the heterogamic yeasts (e.g., *Debaryomyces* and *Nadsonia*), conjugation often occurs between a big mother cell and a smaller cell just budding from it, the large cell playing the part of a female gamete, the tiny one that of a male gamete.

In view of the foregoing discussion we may regard yeasts as heterothallic, and segregation of sex as occurring just previous to conjugation. This is indicated also by our recent researches on *Schizosaccharomyces octosporus* where ascospores are bisexual. If we isolate any one of them in a hanging drop and watch it as it multiplies, it first swells and is then partitioned into two cells, each of which in turn yields by further partitioning a small colony of cells. The latter, when they cease to divide, conjugate between themselves and form asci. Conjugation thus occurs sooner or later, according to the medium, either between cells produced from the ascospores by a great number of successive transverse divisions, or between those with only five or ten generations between the ascospores and themselves. If an ascus from this yeast is placed in an unsuitable medium, the ascospores, being swollen and still contained within the cavity of the ascus, may experience but one partitioning. The two cells obtained through this partitioning conjugate and form an ascus often within the older ascus before it bursts its sheath.

Ascospores, in general, may swell into vegetative cells which conjugate directly without previous multiplication, and the cycle is thus shortened by omission of the vegetative phase. This results from immersing asci into beer brew for a few hours. The ascospores swell then within the ascus and when transferred onto a piece of

plaster of Paris, they divide into two cells which conjugate into a new ascus within the old one. Two ascospores may thus conjugate without previous multiplication. This process cannot be witnessed under the microscope, however, but it is easy to determine whether a particular zygote has arisen from fusion between ascospores or between cells originating from ascospores. This distinction rests upon the fact that ascospores store amyloid material in their walls which stain blue with iodine, but which material is used up as the ascospores divide. Therefore, zygotes resulting from direct fusion of ascospores retain blue-staining material in their walls.

One of the first two mitoses in the ascus must be a reduction division but we have no reason to believe that sexual segregation results from it. Sex does not segregate by cell partitioning, but through an internal change within the initially bisexual ascospore, preparatory to conjugation, and probably due to physiological conditions of the environment.

Besides those forms, just described, which show iso- or heterogamic conjugation just prior to ascus formation, we made known as early as 1903 a number of yeasts wherein conjugation is postponed to the end of the cycle when the ascospores fuse and then germinate, e.g., *Saccharomycodes Ludwigii*. The cells of this yeast multiply by both budding and partitioning, and the asci always yield four ascospores. Previous to germination, these ascospores, having swollen within the ascus, conjugate in pairs through a canal, caryogamy occurring midway. Usually it is the four ascospores of the same ascus which conjugate in pairs. At times, some ascospores may fail to germinate, and conjugation then obtains between two ascospores from neighboring asci. Each resulting zygote immediately germinates into a number of vegetative cells budding from the canal whereby the ascospores fused. The ascus wall meanwhile gelatinizes and the vegetative cells arising from the zygote multiply actively, thus exhausting the nutritive medium until they cease to divide; they then develop into asci. This fusion of ascospores had already been observed in *S. Ludwigii* by Em. Chr. Hansen who, however, failing to study the concomitant cytological phenomena, ignored the sexual implication of the process.

Our researches also demonstrated that ascospores of *S. Ludwigii*, germinating under unfavorable conditions, may conjugate into zygotes which, no longer yielding a succession of vegetative

cells, differentiate directly into asci similar to those formed by *Schizosaccharomyces* and *Zygosaccharomyces*. In such cases the cycle is shortened by the omission of the vegetative phase. We described (18) the same sexual process as occurring also in Johannisberg II yeast, in *Saccharomyces Chevalieri* and *S. Mangini*, in a species of *Saccharomyces* isolated from fermenting Mexican pulque, and in *Hansenula Saturnus*. The process is widespread and has been found also in *Saccharomyces ellipsoideus*, *S. intermedians*, *S. validus*, *S. turbidans*, *S. Williamus*, *S. Bayanus*, *S. vini*, *S. Muntzii* (34) and in *S. annulatus* (36); also in twelve strains of *Saccharomyces* isolated from pears (4), and in sixteen strains of *Saccharomyces* isolated from white wines at Pouilly-sur-Loire in the Nièvre department (37).

Winge (47a) and Lausten (33a), independently, have recently demonstrated that *Saccharomyces Ludwigii* actually is heterothallic. Using a micromanipulator to isolate the cells, and germinating each singly, they showed that not all the cells behaved alike, some germinating into elongated cells, some into short cells, others into a number of haploid cells, and still others yielding very few cells since they soon ceased to multiply. The Danish authors explained this behavior by postulating the existence of four genes, *N*, *l*, *n* and *L*, one being a factor for growth, another inhibiting growth, a third being a factor for short cells and the fourth a factor for long cells. One of the two ascospores in each end of the ascus, having the genes *N* and *l*, the other *n* and *L*, the zygote formed by fusion of these two spores thus receives the four genes. Mlle. Manuel used a "microdissecteur" to isolate the four ascospores from single asci and almost constantly obtained conjugation by mating the two ascospores of each pair. By mating spores of different pairs she obtained 50% conjugation. Conjugation took place only between ascospores, as such, and never between haploid cells developing from isolated ascospores. As soon as an ascospore began to germinate it lost its sexual potentiality and therefore with its ability to conjugate.

PARTHENOGENESIS

Besides asci resulting from iso- or heterogamic conjugation, parthenogenetic asci are formed as a rule in *Zygoichia* and *Zygosaccharomyces*, whereas in *Schizosaccharomyces* such development is exceptional. As a rule, the ascus-forming cell, which may be

considered as a parthenogenetic gamete, develops one or many lateral tubes, as if attempting to unite with another gamete, before developing ascospores. We even observed one species, *Zygosaccharomyces Pastori* (16), wherein conjugation was laboriously achieved by gametes which displayed many vain attempts to unite by means of several protuberances before they finally succeeded. Asci resulted more often, however, from parthenogenesis than from conjugation. Finally, in *Schwanniomycetes* and *Torulospora* parthenogenesis becomes the rule, although spore-forming cells always vainly attempt to fuse with others by producing abortive tubes.

There is an important difference between *Saccharomycodes Ludwigii* and species of the genus *Saccharomyces*. The ascospores of the former conjugate, as a rule, parthenogenesis being exceptional, whereas in *Saccharomyces* the proportion of ascospores which conjugate varies from 50% in certain species to 25% in others; the non-conjugating ascospores germinate by themselves. The same applies to *Hansenula Saturnus* where conjugation is difficult and the asci yield from one to four ascospores. When there are one or three ascospores conjugation is possible only when the asci are so crowded together that ascospores from neighboring asci can conjugate. We were obliged, therefore, to recognize a tendency toward parthenogenesis in *Saccharomyces* and in *Hansenula Saturnus*.

HAPLOBIONTIC AND DIPLOBIONTIC YEASTS²

As far as sexuality is concerned, the foregoing results require that the following two types of yeast be recognized:

1. The haplobiontic, wherein iso- or heterogamic conjugation precedes ascus formation (*Schizosaccharomyces*, *Zygosaccharomyces*, *Zygopichia*, *Debaryomyces*, *Nadsonia*, *Nematospora*).

2. The diplobiontic, wherein conjugation is postponed until later in the cycle, ultimately to occur between ascospores about to germinate (*Saccharomyces Ludwigii*, *Hansenula Saturnus*, and numerous other species of *Saccharomyces*).

² It should be clearly understood that we shall here use a terminology somewhat different from the too complicated one evolved by Svedelius. We shall term "haplobiontic" any plant where meiosis takes place as soon as the zygote germinates, the diploid phase, therefore, being restricted to the zygote. We shall term "diplobiontic" any plant where meiosis immediately precedes gamete formation, the haplophase being then restricted to the gametes. Finally, we shall regard as "haplo-diplobiontic" any plant displaying an alternation of generations, *i.e.*, having a diplophase and haplophase.

Because of the small size of the nucleus, it has not been possible to study nuclear reduction. However, we have observed mitoses in the ascus of *Schizosaccharomyces octosporus* (21), a most favorable material. It is similar to that in the ascus of higher Ascomycetes, but the chromosomes are too small to be counted. Recently, however, Badian reported that he was able to follow nuclear reduction in one yeast and that the number of chromosomes was two. His figures, however, are too diagrammatic to be taken into consideration; they fit neither with our nor with his own photomicrographs.

Since in any higher Ascomycete, where mitoses can be minutely investigated in the ascus, nuclear reduction is seen to occur at one of the first two mitoses, we were led to believe that in yeasts, also, meiosis must occur within the ascus. Therefore, the two categories mentioned above differ widely in their nuclear evolution. Where conjugation occurs before ascus formation, the diplophase is restricted to the zygote, while the entire development, from ascospore to zygospor, belongs to the haplophase. Therefore, such yeasts are haplobiontic. On the contrary, among yeasts where ascospores conjugate, the diplophase takes up the whole cycle from the zygote, resulting from fusion between ascospores, to the ascus, while the haplophase is restricted to the ascospores. The cycle of development is, therefore, diplobiontic, the opposite of that in the other yeasts.

We have already noted that under unfavorable conditions either group may omit the vegetative phase; among haplobiontic forms, ascospores may conjugate, omitting the vegetative haplophase; conversely, among diplobiontic forms, the zygote, originating by fusion of two ascospores, may forthwith differentiate into an ascus, omitting the vegetative diplophase. Conjugation thus may take place at the same stage in both groups. Such cases, however, are exceptional and of no importance, but they show that the vegetative cycle is not indispensable. Finally, parthenogenetic forms may develop in both groups.

DERIVATION OF THE HAPLOBIONTIC YEASTS FROM THE ENDOMYCETACEAE

Our researches (16) on the Endomycetaceae, a group of Ascomycetes which was scarcely known at the time, led us to trace the relationship between them and yeasts. The Endomycetaceae are lower

Ascomycetes, building their vegetative parts from uninucleate cells assembled into mycelia. Among them, *Eremascus fertilis* was studied by Mlle. Stoppel and by ourselves, and was shown to produce gametes from neighboring cells of the mycelium, which gametes conjugate (isogamy) by pairs into asci bearing eight ascospores. Besides this fungus, the family includes other genera, such as *Endomycopsis*, wherein the mycelium propagates by budding yeast-like cells which show the same aspect and structure as yeasts. These cells can multiply for a while by budding until conditions become favorable for mycelial growth. Other species, as those of the genus *Endomyces*, never bud in this yeast-like manner but multiply through cells breaking loose from the mycelial threads. Thus set free, these cells, termed oidia, propagate by septation before developing a mycelium. These are strictly comparable to the cells of *Schizosaccharomyces*. Asci, similar to those of yeasts, are formed on certain parts of their mycelia. The ascospores within the asci may display some of the shapes observed in certain yeasts, hat-like with a protruding rim (*Hansenula*), or spiny-walled and with a protruding band (*Schwanniomyces*). The asci generally proceed from iso- or heterogamic conjugation, as in yeasts, and the resulting zygote differentiates directly into an ascus, bearing four ascospores.

Therefore, the Endomycetaceae are haplobiontic as are some of the yeasts. Certain species, however, develop parthenogenetic asci from gametes which vainly attempt to unite with one another, e.g., *Endomycopsis* (*Endomyces*) *fiduliger* and *E. Lindnerii*, while other species no longer display any sexual tendency, e.g., *Endomyces decipiens* and *Endomycopsis capsularis*. On the whole, they are closely similar to yeasts, differing from them only in their forming typical mycelia which, however, spread by means of yeast-like cells (conidia), or oidia similar to cells of *Schizosaccharomyces*. Moreover, the display of sexuality preceding formation of their asci is quite similar to that in yeasts.

It is most easy, therefore, to derive yeasts from endomycetaceous fungi by assuming a regression to such degree that the ability to develop mycelia has been lost and that the vegetative phase has become restricted to cells which propagate by budding, or to oidia which divide by septation. Such changes have naturally involved sexual differentiation which could not obtain between cells of a mycelium but which developed between the yeast-like cells or be-

tween oidia. A number of intermediary forms grade from the Endomycetaceae down to the yeasts. In *Endomycopsis* (*Endomyces*) *javanensis* and *E. (Guilliermondia) Selanosporus*, for instance, the mycelium is very restricted, and yeast-like cells may develop into asci as well as into mycelial cells. *E. javanensis* was considered as being non-sexual, whereas Nadson and Konokotine attributed isogamic conjugation to the other species. The latter observation was subsequently confirmed by Mlle. Manuel (31) who recently claimed that *E. javanensis* also develops asci through isogamic conjugation between two mycelial cells. Moreover, we know of yeasts (*Zygosaccharomyces Marzianus* (25), *Debaryomyces Klöckeri* (27) and *Nematospora Coryli*) which under certain conditions may produce well developed mycelia from which asci may develop. In fact, most yeasts may produce at least rudimentary mycelial mats on aging, and it is very difficult to distinguish definitely between them and the Endomycetaceae.

The theory concerning the phylogeny of yeasts that we published in 1909, due consideration being given to the hopeless task of distinguishing the Endomycetaceae from the Saccharomycetaceae, was the foundation on which Frau Stelling-Dekker (43) founded her wise decision to combine both families into one, the Endomycetaceae, which in turn was subdivided into two subfamilies. The genus *Eremascus*, formerly included in the family Saccharomycetaceae, was placed in its own family, the Eremasceae. Of the two subfamilies, that of the Endomycoidaeae includes, under the same generic name, those species of *Endomyces* which have oidia, and the genus *Schizosaccharomyces*. The other subfamily, Saccharomycetoidaeae, includes, first, all the other forms formerly included in *Endomyces* which propagate through yeast-like conidia, now renamed *Endomycopsis* by Frau Stelling-Dekker; secondly, all the budding yeasts. The latter may have been derived from the former.

DERIVATION OF DIPLOBIONTIC YEASTS FROM THE EXOASCALES

Until recent years no Ascomycete was known to indicate relationship with the diplobiontic yeasts, where conjugation occurs between ascospores, and these yeasts, therefore, stood alone, their phylogeny remaining most obscure. The results of our researches on haplobiontic yeasts had been quickly integrated into the classical heirloom of knowledge, but because of this deficiency just noted,

our results pertaining to diplobiontic forms were not accepted without reserve, in spite of all the care we had taken in substantiating the caryogamy attending the conjugation of ascospores. It was particularly from Dangeard (5) and later from Kniep (28) that objections were encountered.

Since then, new facts, bearing on sexuality among the Exoascales, have somewhat illuminated the matter. Long ago, Dangeard showed that in these fungi, often considered as closely related to yeasts, an ascus-producing cell always contains two nuclei, and that within the young ascus these nuclei undergo a caryogamy, credited by that scientist with sexual significance. This caryogamy, as found in the asci of all higher Ascomycetes, divorces the Exoascales from the Endomycetaceae and yeasts, and led many botanists to ascribe the Exoascales to the higher Ascomycetes. In addition, Fräulein Wieben (444) followed the development of two species of Exoascales, *Taphrina epiphylla* and *T. Klebanhii*, in pure culture, and showed that the ascospores germinate into haploid yeast-like cells which soon conjugate into zygotes. Two yeast-like conidia fuse through a canal whereby one discharges its contents into the other, but caryogamy does not occur with the mixing of the cytoplasm.

This anomalous situation is common to all the higher Ascomycetes and to the Basidiomycetes, both nuclei, male and female, instead of fusing, co-existing in the zygote as a couple of nuclei constituting what is known as a dikaryon. The zygote containing the dikaryon immediately germinates into a mycelium wherein both nuclei of the dikaryon simultaneously divide at each cell division. Such divisions constitute "conjugate mitosis" whereby the mycelium is built up of binucleate cells, each containing a male and a female nucleus. Toward the end of vegetative development, asci form on the mycelium, and the binucleate cells which give rise to them then display the caryogamy described by Dangeard. The resulting diploid nucleus undergoes two successive mitoses, one of which is the reduction division.

Therefore, the Exoascales, as studied by Fräulein Wieben, show an alternation of generations with, first, a very short haploid phase restricted to the ascospores and to the yeast-like conidia proceeding from them; secondly, a diploid phase which begins with the zygote and ends when ascospores are formed. This latter phase covers practically the entire development of the fungus, being "a dicaryotic

haplophase." Instead of involving cells each of which bears a single diploid nucleus (*i.e.*, a nucleus enclosing two n chromosomes: n male + n female chromosomes within its nuclear membrane), it involves cells having two " n -chromosome nuclei," one nucleus a male, the other female. Caryogamy just precedes reduction division.

The homology between this cycle of development and that of diplobiontic yeasts can easily be conjectured, and, as early as 1931, we stressed (23) the relations which might bind the diplobiontic yeasts to the Exoascales as studied by Fräulein Wieben. Barring the dikaryon, the great similarity between these two groups of Ascomycetes is obvious, the main difference being that, among the Exoascales, conjugation does not operate immediately between ascospores, as it does among diplobiontic yeasts, but between cells that issue from their germination, a short haploid phase intervening. The Exoascales, therefore, are not strictly diplobiontic, but the haplophase in their alternation of generations is so restricted that they appear to be so.

This problem pertaining to those yeasts wherein conjugation operates between ascospores was recently promoted through a new avenue which again accentuated the relationship we have suggested as existing between these yeasts and the Exoascales. In various species of *Saccharomyces* (*S. ellipsoideus*, *S. cerevisiae*, *S. validus*, *S. intermedius*, *S. turbidans* . . . Johannisberg II yeast and *S. pastorianus*), Kruis and Satava (29), then Satava (42), found that conjugation may occur either between ascospores, as we had described, or later between the first haploid cells budding from them. Therefore, direct germination of the ascospores without previous conjugation does not imply that they develop parthenogenetically, as we had assumed. We had neglected to follow the destiny of the cells as they budded from ascospores, not foreseeing that they might conjugate later. At that time we did not recognize, as is so well known today, that the sexual act may occur at different phases in the life cycle of a given fungus.

The papers of Kruis and Satava, being published in Czech, remained all but unknown and were not reported on until recently when Winge (45), having independently attained the same results from various species of *Saccharomyces* (*S. ellipsoideus*, *S. validus*, *S. Marchalianus*, Johannisberg II yeast), quoted the Czech authors

at the end of his paper. Winge reported a difference in size and shape between haploid cells budding from ascospores and diploid cells budding from the zygote; as a rule, the former were spherical, the latter elongated and larger. All the species investigated were found to be homothallic, and any single ascospore could yield haploid cells which conjugated into zygosporos.

Winge's paper, as read before the International Botanical Congress at Amsterdam (1935), suggested that we reinvestigate the yeast (*Saccharomyces paradoxus*) discovered by Mlle. Batschinskaia in which she had reported a most inconceivable cycle of development, including two sexual acts in succession (8), first, conjugation between ascospores, second, conjugation between the first cells to proceed from the zygote which was assumed to have resulted from fusion between two ascospores. The zygotes, as obtained through the second conjugation, then appeared to yield vegetative cells which would multiply actively, later to differentiate into asci. This was inexplicable, *a priori*, having no precedent among living organisms.

On resuming the study of that yeast in 1931 we observed ascospores to conjugate as they germinated, but throughout the development of the resulting zygotes we could never observe the second division as reported by Mlle. Batschinskaia. We had neglected, however, to trace the development of those ascospores which germinated without first conjugating, but after Winge's discovery, it appeared worthwhile to follow the developmental cycle of that yeast, an easy task, for its asci form profusely. About 7 P.M. in the evening we isolated some in hanging drops, in moist Van Tieghem and Le Monnier cells, and duly checked their places. Next morning, the ascospores, one to four in each ascus, were found to have swelled within the asci, previous to germination, and, as germination began, the ascus wall gelatinized. When swollen the ascospores adhere together closely and also to the wall of the ascus itself. Therefore, when the ascospores number four or three, they show a polyhedral contour; when only two, their common face appears like the transverse septation in a dividing cell. Germination occurs during the day, which makes it easy to follow all stages under the microscope. These ascospores were found to conjugate directly into zygotes which budded actively, but most of them germinated without conjugating, yielding haploid cells, clumped to-

gether in small colonies. Conjugation ultimately occurred, however, either between haploid cells formed by the budding of one or more ascospores from the same ascus (sometimes from neighbouring asci) or between haploid cells issuing from one ascospore. That this yeast is homothallic is easily noted by watching the germination of the ascospores of a one-spored ascus when only haploid cells are formed to conjugate between themselves. Though conjugation usually seems to occur between the first haploid cells budding from the ascospores, it may take place at various stages, between cells resulting from four to fifteen successive buddings, or between the first two buds from the ascospore, or even between the ascospore itself and the first cell budding from it.

Regardless of whether the zygotes are the results of conjugation between ascospores, or between haploid cells budded from the ascospores, they immediately germinate to yield a number of diploid cells. These propagate for a while by budding to form asci until the medium becomes exhausted. Some haploid cells appear never to conjugate; they are probably the same sporogenous cells as one observes in old cultures. No difference was noted between the haploid and diploid cells though the latter may be somewhat bigger.

How long diploid cells resulting from germination of zygotes will multiply depends on the medium. In certain cases propagation may be curtailed to just a few cells which soon differentiate into asci, and on non-nutrient agar (Gorodkova) or in a moist chamber zygotes yield only a few cells clumped in small colonies where budding is soon inhibited as sporulation intervenes.

Immersion of asci of *S. paradoxus* in beer-must for about twelve hours, so that the ascospores may swell and build up reserves, and subsequent transference to a piece of plaster of Paris, induces a shortening of the cycle. Each of the swollen ascospores, ungerminated as they are removed from the must, yields one or two haploid cells which conjugate immediately either with one another or with the ascospores which give rise to them. The resulting zygotes, being starved, use the reserves built up by the ascospores as they swell in the must and yield a few cells, sometimes only one, which later differentiate into asci. The zygote itself, since it results from conjugation between ascospores or between derived haploid cells, may develop directly into an ascus without previous budding, and in such an event the ascus displays a bulge at each end of the canal, as was featured above for *Zygosaccharomycodes*. We have

noted this situation also in *Saccharomycodes Ludwigii* where the haploid phase is so shortened as to be omitted and the diploid vegetative phase is restricted to a few cells or even omitted altogether. Conversely, in haplobiontic yeasts, such as *Sch. octosporus*, the vegetative phase, now the haploid, may be restricted to a few cells, or omitted.

So, just as among the yeasts investigated by Kruis and Satava and by Winge, conjugation in *S. paradoxus* sometimes obtains between the ascospores as such but more often between the first haploid cells budding from them. It was here that Mlle. Batschinskaia erred, for she interpreted two different methods of accomplishing one sexual act, as two successive sexual acts.

From our observations regarding *S. paradoxus*, from those by Kruis and Satava, and from those by Winge on various species of *Saccharomyces*, it may be concluded that a whole series of yeasts belonging to the genus grades off from the haplobiontic yeasts (*Zygosaccharomyces*, *Zygopichia*, *Debaryomyces*, *Nadsonia*, *Nematospora* . . .) where conjugation just precedes ascus formation, to the diplobiontic yeasts where conjugation operates only between ascospores. In *Saccharomycodes Ludwigii* we recently found again that conjugation takes place only between ascospores, and that those ascospores which may germinate singly are actually parthenogenetic. That yeast is, therefore, typically diplobiontic.

In intermediate forms conjugation operates eventually between ascospores as such, though more often between haploid cells resulting from ascospores through budding; these yeasts, therefore, generally show an alternation of generations, for between ascospore formation and cell conjugation, a few vegetative cells build up the haploid phase.

We may wonder how far the results of such researches may be treated as general.

From our earlier research it was learned that conjugation between ascospores occurs not only in the Johannisberg II yeast, belonging to the genus *Saccharomyces*, but also in a very different yeast, *Hansenula Saturnus*; our more recent researches and those conducted by our students Marchand, Negroni, Capitain and Renaud, revealed such conjugation among many species of *Saccharomyces*. A reinvestigation regarding the sexuality of the different genera of yeast proved necessary, and we induced one of our students, Mlle. Manuel (32, 33), to undertake the problem. From as yet unpublished data

(a few notes concerning the most salient results have appeared) it may be inferred that most species of *Saccharomyces* behave as reported above. That is, in *S. Mangini*, *Chevalieri*, *Lindnerii*, *Mangini* var. *tetrasporus*, *Williamus*, *turbidans*, *Bayanus* and *cerevisiae* var. *alpina* conjugation may operate between ascospores as such, although in more than half the instances we find it taking place between haploid cells arising from ascospores which have budded by themselves.

Haploid cells, as they proceed from ascospores by budding, may often be distinguished by their spherical shape from elongated diploid cells budding from zygotes (Winge). Rather different, however, is the behavior of the Logosse yeast, where conjugation was never observed between ascospores as such, but only between haploid cells derived from them by budding. To conclude, all these species, as investigated, are homothallic. Thus, this type of sexuality between ascospores or between the haploid cells which derive from them, may appear in any form of *Saccharomyces* and characterizes that genus. This, however, does not preclude some forms from being parthenogenetic. A profusely sporogenous strain of *Saccharomyces*, for instance, which J. Renaud isolated from wine, displayed conjugation only for 10% of the ascospores, most of which germinated by themselves, yielding cells which never conjugated though they may have formed asci. Renaud (41) transferred swollen cells of this yeast from beer-must to plaster of Paris, and watched them differentiate directly into asci without conjugation. The species, therefore, tends toward parthenogenesis. Conjugation could still operate at any stage between its haploid cells issuing from the germinated ascospores, and the objection may be raised that conjugation might have occurred without having been detected. To answer that objection, J. Renaud took care to keep some ascospores in beer-must long enough to swell, but not to germinate; he then starved them by transferring them to pieces of plaster of Paris. Swollen but still within the ascus, they did not bud, but each differentiated into a new ascus without conjugation, and parthenogenesis was thus demonstrated.

Conjugation similar to that just reported was observed by Mlle. Manuel also in *Hansenula Saturnus* and *H. margaritae*, though she did not note it in *H. anomala* var. *sphaerica* or in *H. javanica* which may, therefore, be considered parthenogenetic.

As to species of the genus *Pichia*, they behave in an interesting manner. Among those which Mlle. Manuel investigated, *P. membranifaciens* var. *calliphora* displayed conjugation between ascospores as such or between resulting haploid cells; the others, *P. Chodati* and *P. Trumpyi*, showed conjugation prior to ascus formation, and, therefore, belong to the genus *Zygopichia*. *P. Mandshurica*, showing no sexuality, is parthenogenetic.

HAPLOBIONTIC-DIPLOBIONTIC HYBRIDS

In a paper published later than the aforementioned, Winge and Lausten described their use of a micromanipulator in isolating ascospores from an ascus of a certain strain of *Saccharomyces ellipsoideus*. Each ascospore cultivated alone germinated into spherical haploid cells which conjugated with one another, giving rise to zygotes. These zygotes germinated yielding elongated diploid cells some of which developed into asci when grown on pieces of plaster of Paris, where haploid cells would never sporulate. However, some of the germinating ascospores yielded elongated cells directly, similar to the diploid cells. They were able to differentiate into asci when placed on pieces of plaster. The authors postulate that from the very beginning such ascospores contained two nuclei which fused when germination occurred. Winge and Lausten (46, 47) isolated the four ascospores from the same ascus and caused each to form a giant colony. Each colony differed morphologically from the others and thus disclosed that the zygote was heterozygous. As it differentiated into an ascus, meiosis segregated the factors among the four ascospores, following Mendel's laws.

The same authors isolated ascospores from baker's yeast (type related to *S. ellipsoideus*) and from *S. validus*. They deposited side by side one ascospore of the former and one of the latter in the same hanging drop and obtained a hybrid zygote which germinated into diploid cells. That hybrid displayed a type of cell morphology and a manner of growing intermediate between those of the parents, although closer to the baker's yeast.

CYTOLOGICAL FEATURES AT CONJUGATION AMONG HAPLO-DIPLOBIONTIC YEASTS

It is now fitting to recall a fact which we reported in our older studies but without due emphasis, a fact which is brought into prominence by what we have just said regarding the independence

in the zygote between caryogamy and budding. In *Saccharomyces Ludwigii* caryogamy occurs at conjugation, but that is not true for other yeasts. In Johannisberg II yeast we showed that caryogamy may occur during fusion of the gametes, as in *S. Ludwigii*, but that it may be deferred to the time when the zygote buds. The first bud may attain adult size before the two nuclei fuse within the zygote, and the diploid nucleus thus obtained through caryogamy immediately divides. It first elongates, constricting in the middle, and shows all the appearance of amitosis. In *Hansenula Saturnus*, however, we observed mitosis-like nuclear features.

There are cases, on the other hand, wherein the two haploid nuclei, coexisting within the zygote, fail to conjugate. They divide synchronously showing "conjugate division" into daughter nuclei which fuse within the bud. This occurs in *S. paradoxus*, and it may be interpreted as a tendency toward the dikaryon which we always find in the higher Ascomycetes. We suggested this in 1911 (7). More recently, Nadson and Konokotine demonstrated nuclei to coexist as dikaryons in the female gametes of *Nadsonia*, and to migrate as such into the ascus budding off the gamete, only there to show caryogamy. One of our students, J. Renaud (39), observed features in a wine yeast of the *S. ellipsoideus* type which were quite similar to but more distinct than those reported above for Johannisberg II yeast or *S. paradoxus*. A zygote, having no caryogamy but two nuclei coexisting dikaryon-like, displayed "conjugate mitoses" and budded into two or three catenated diploid cells, each with two nuclei, the second or third cell being the seat of the postponed caryogamy. Here was actual development of a dikaryon in a yeast. Renaud (40) also observed an anomalous condition by transferring a newly formed zygote with as yet unfused nuclei into a fresh medium in a moist chamber. The zygote then budded profusely and some of the daughter cells received only one haploid nucleus. Thus the two nuclei of the dikaryon were divorced and the resulting haploid cells soon conjugated into new zygotes. This anomaly, most rarely obtainable, might in part explain Mlle. Batschinskaja's misinterpretation in the case of *S. paradoxus*.

As to nuclear division at budding, our recent researches on *S. paradoxus* have revealed mitosis-like features. Recently, Badian (1) featured nuclear division in this yeast as involving two chromosomes at mitosis, but, as we have already explained, that author's interpretation can scarcely be accepted. Renaud's (38) recent rein-

vestigation failed to confirm Badian's view, for they indicated that nuclear division took place through mitosis, involving an achromatic spindle connecting at each end with a small centrosome. Each centrosome is at first located where budding occurs, but then migrates, towing the mitotic figure into the bud. The chromosomes are so small that they cannot be accurately counted.

RECENT STUDIES ON THE PHYLOGENY OF HAPLO- AND DIPLOBIONTIC YEASTS

Kruis and Satava as well as Winge have failed to realize that the facts reported above strongly uphold our theory as proposed in 1931 concerning the relations between diplobiontic yeasts and the Exoasceae. We pointed out that, except for the dikaryon, the cycle of development in diplobiontic yeasts was regarded as differing from that of the Exoascales only in their conjugation occurring between ascospores as such rather than between haploid cells derived from budding of ascospores as in the Exoascales. This difference, we now know, is true only of *Saccharomyces Ludwigi*, for the other yeasts, the diplobiontic forms, most often do not display conjugation between ascospores as such, but between the first haploid cells budding from them. They thus act like the Exoascales studied by Fräulein Wieben.

On the other hand, the above reported cytological features demonstrate that these yeasts may form zygotes without caryogamy, the two nuclei coexisting within the zygote as a dikaryon and dividing simultaneously to yield a number of binucleate cells before fusion occurs.

These facts confirm the idea of a relationship which we suggested as existing between the diplobiontic and haplodiplobiontic yeasts, on the one hand, and *Taphrina* (*T. epiphylla* and *T. Klebanhii*) on the other. Thus the Exoascales, which most mycologists still separate from the yeasts so as to bring them nearer the higher Ascomycetes because of caryogamy within the ascus, appear to us, on the contrary, much closer to yeasts than was assumed, so that we have to revert to the earlier notions of de Bary and Hansen.

CONCLUSIONS

Thus we see that our present knowledge of yeasts indicates that they belong to three types, quite different in their developmental cycles:

1. The haplobiontic (*Schizosaccharomyces*, *Zygosaccharomyces*, *Zygopichia*, *Debaryomyces*, *Nadsonia*, *Nematospora*, *Coccidiascus*), closely related to the Endomycetaceae.

2. The diplobiontic (*Saccharomycodes Ludwigii*).

3. Those which tend to be diplobiontic and may indeed be so because they generally display an alternation of generations through a very restricted haplophase. They are, therefore, haplo-diplobiontic (*Saccharomyces*, *Hansenula*, *Pichia membranifaciens*).

The diplo- or diplo-haplobiontic cycle of the second and third types is to be compared with that of certain Exoascales (*Taphrina epiphylla* and *T. Klebanhii*), and the yeasts displaying such cycles may be considered as more or less closely related to them.

Haplobiontic yeasts undoubtedly have been derived from the Endomycetaceae with which they retain close relations.

A much looser relationship may be conceived between yeasts and the Ustilaginales which many mycologists place near the Exoascales with which yeasts are affiliated. Quite different from the Exoascales and belonging to the Basidiomycetes, the Ustilaginales, nevertheless, resemble the Exoascales in yielding a succession of cells so yeast-like as not to be distinguishable from true yeasts. Moreover, the Ustilaginales, according to Rawitscher, Kniep and others, are the only other fungi which exhibit the developmental cycle characteristic of the yeasts and the Exoascales.

Among the Ustilaginales, chlamydospores fulfill the functions of asci and may be considered homologous to the asci of the Exoascales, for they first show two nuclei which later fuse through caryogamy. The resulting diploid nucleus undergoes two mitoses in succession, one being the reduction division; then the chlamydospore germinates into a short mycelium, termed a promycelium, into which the four haploid nuclei migrate, each to be allotted to one of the four uninucleate haploid cells into which the promycelium is divided by two transverse septations.

Among the Ustilaginales three types of development are homologous with three types, respectively, in the yeasts. Some species, such as *Ustilago carbo*, are diplobiontic, like *Saccharomycodes Ludwigii*, two conjugations among the four cells of the mycelium resulting in two zygotes, each containing a dikaryon like the zygote among the Exoascales. Each zygote germinates into a diploid mycelium, made up of binucleate cells, featuring a dikaryotic diplophase, such as occurs in the Exoascales, and ultimately yielding chlamydospores

wherein caryogamy occurs. Others, such as *Ustilago violaceum*, behave as do species of the genera *Saccharomyces* and *Taphrina* (*T. epiphylla* or *T. Klebanhii*). In them alternation of generations includes a haploid phase restricted to a few cells; caryogamy operates between external spores, the so-called sporidia, issuing from each cell of the promycelium, or between yeast-like conidia budding from each of the former cells. Still others, such as *Ustilago Maydis*, are haplobiontic, conjugation operating just prior to chlamydospore formation. Development in these species is similar to that in the Endomycetaceae, in haplobiontic yeasts, and in *Taphrina deformans*.

Without stressing the similarity in development between yeasts and the Ustilaginales which discloses the close relationship recognized by all mycologists between Ascomycetes and Basidiomycetes, our understanding of the cycle of development in yeasts may be considered today as definitely clarified. Recent works discussed in this paper have shed light on the phylogeny of yeasts which had long been solved with respect to haplobiontic forms but which was incomplete regarding diplobiontic forms. These, in the light of recent research (*Saccharomyces Ludwigii* being excepted), do not appear strictly diplobiontic, but show an alternation of generations with a very short haplophase. Their cycle of development, therefore, is quite comparable to that of certain Exoascales (*Taphrina epiphylla* and *T. Klebanhii*) to which they are more or less closely related, for recent studies have revealed relationships between the Endomycetaceae and the Exoascales. Diplobiontic and haplo-diplobiontic yeasts, on the other hand, do not evidence such direct parentage with the Exoascales.

The Exoascales represent among the lower Ascomycetes a more highly developed group than do the yeasts, for by their dicaryotic diplophase they stand nearer the higher Ascomycetes.³ They may

³ According to Marin, *Taphrina deformans* yields bisexual ascospores which germinate into bisexual yeast-like conidia which fail to conjugate and grow a mycelium. The dicaryotic phase is assumed to precede formation of the ascus, and to occur within the host as two mycelial cells fuse. Were this true, this species would then be haplobiontic, like the Endomycetaceae (see Kniep: Die Sexualität der niederen Pflanzen). However, newer researches by Mix seem to indicate that dicaryons obtain within conidia, as a rule, through nuclear division, or, exceptionally through two nuclear divisions or through fusion of two conidia. *T. deformans* should, therefore, be considered diplobiontic, as *T. epiphylla* and *T. Klebanhii* are regarded.

be considered as lower Ascomycetes derived from the Endomycetaceae by representing a higher type of development than they.

It appears plausible, in final conclusion, that the yeasts as a group have developed from forms similar to the Endomycetaceae. Some appear to have remained haplobiontic, like the Endomycetaceae, whereas others, evolving like the Exoascales, to which they are related in the same way as to the Endomycetaceae, have become diplobiontic or at least tend to be so.

Editor's Note: The foregoing article involves, in part, a terminology which appears to cause confusion in the minds of many readers. In an effort to clarify this matter, Dr. B. O. Dodge was invited to formulate the following, which he has kindly done:

Haplobiontic and diplobiontic organisms. Considerable confusion has resulted from attempts of investigators to use the terms *haplobiontic* and *diplobiontic*, originally proposed by Svedelius in his paper "Zytologisch entwicklungsgeschichtliche Studien über *Scinaia furcellata*, ein Beitrag zur Frage der Reduktionsteilung der nicht tetrasporenbildenden Florideen," Nova Acta Reg. Soc. Sci. Upsala 44: 1915. Haplobiontic species, according to Svedelius, are those like *Nemalion* and *Fucus* which complete their entire life cycle as one plant. The former is haploid throughout the vegetative stage but the latter is diploid. Nevertheless, they are both haplobiontic. A diplobiontic species, on the other hand, is one like *Polysiphonia* or *Dictyota* where the complete life cycle involves two different plants. Whether a phase is haploid or diploid is not a criterion in determining whether a species is haplobiontic or diplobiontic. The question to be answered is whether there are *two kinds of plants or only one*.

Among the yeasts, *Saccharomycodes Ludwigii* is normally haplobiontic, although the vegetative stage consists of diploid buds or cells. *Schizosaccharomyces octosporus* is also haplobiontic, although its vegetative stage is composed of haploid chains of cells. *Saccharomyces ellipsoideus* is diplobiontic but only to the extent that ascospores germinate and form a number of rather small round budding cells; later, some of these cells fuse in pairs. From here on the budding yeast cells are larger and more ellipsoid. The fact that the small round budding cells of the first phase happen to be haploid while the larger ellipsoid cells of the second phase are diploid, is not the factor which renders this species, to a certain extent, diplobiontic. If the ascospores on germinating fused in pairs to produce a diploid generation, the species would then be haplobiontic.

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VERNALIZATION AND THE GROWTH-PHASE CONCEPT

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INTRODUCTION

The term vernalization is the anglicized form of the Russian word iarovization. It signifies that certain winter annuals and biennials can be induced to follow the spring-annual habit by suitable treatment of the germinated seed or the active bulbs before planting, thus making it possible to obtain sexual reproduction the first season from spring plantings. However, as the term is now used by some workers it embraces practically all of the environmental factors and all the methods applied at any time in the plant's development and which are capable of accelerating sexual reproduction in any species of plant. As the term iarovization was first used it referred to treatments which altered certain germinated seeds physiologically, these alterations in turn changing the direction of growth in suitable subsequent environments. The term was not applied to the breaking of a rest period as in the potato and many seeds. However, there has been a tendency among some workers to apply the term vernalization in this connection. In fact, it has been applied by Shchernetsky (63) to the process of soaking sugar beet seed in water for 4 days at 17.2° to 21.1° C. before planting with the intention of increasing the tonnage and sugar content of the roots.

In general it may be stated that the fundamental concept or principles of vernalization are based on the following facts: (1) that species and varieties possess different optimum environmental requirements during their several critical growth phases, (2) that these optimum requirements must be met within certain limits, otherwise sexual reproduction will not occur or it will be delayed, and (3) that certain of these environmental requirements which are naturally supplied during the first developmental phases in certain plants can be supplied artificially to the slightly germinated seeds or to bulbs before planting.

HISTORICAL BACKGROUND

In the present article reference will be made to those papers which appear the most pertinent, and no attempt will be made to

cite all of the many titles in the literature. However, an attempt is made to cite papers which have good literature lists. These taken together with the present list should enable any student to become acquainted with the entire field very easily. Papers having rather large literature lists are so indicated in the literature list of this paper.

During recent years, Lysenko (42, 43, 44, 46), a Russian investigator, has devoted much time to the study of vernalization and growth phases in many crop plants. In Russia this line of physiology is being carried forward on a very large scale; however, it seems only fair to point out that the basic concepts (54, 70, 71) involved in these studies have been known within certain circles for many years. They simply have not been recognized in all circles of plant science until recently.

The older horticultural and agricultural journals and the older scientific journals and text books show that some growers and some botanists recognized part or all of these concepts. To take the specific phenomenon, vernalization of wheat, on which Lysenko developed his views, it is found that in 1857 Klippart (35), an early observer and close student of crops who was associated with the Ohio State Board of Agriculture, published a rather clear elucidation of this phenomenon in one of his annual reports to the State Board. However, 20 years previous to Klippart's record it was recorded that a grower in New York had produced a crop of grain from spring-sown winter-wheat seed which had been subjected to low temperatures before seeding. In 1850 Allen (1), an agricultural expert of that day, recorded a similar observation. It appears, therefore, that in Klippart's time the idea of vernalization was more than local in the United States. Since Klippart's record is so clear and to the point it is quoted as follows:

"To convert winter into spring wheat, nothing more is necessary than that the winter wheat should be allowed to germinate slightly in the fall or winter, but kept from vegetation by a low temperature or freezing, until it can be sown in the spring. This is usually done by soaking and sprouting the seed, and freezing it while in this state and keeping it frozen until the season for spring sowing has arrived. Only two things seem requisite, germination and freezing. It is probable that winter wheat sown in the fall, so late as only to germinate in the earth, without coming up, would produce a grain which would be a spring wheat, if sown in April instead of September. The experiment of converting winter wheat into spring wheat

has met with great success. It retains many of its primitive winter wheat qualities, and produces at the rate of 28 bushels per acre."

In 1883 Hellriegel (26) concluded that barley has a lower optimum temperature during the tiller-formation phase than during the stem-elongation phase.

In 1893 the phenologists, ecologists and horticulturists met with the International Meteorological Congress in Chicago and their several papers were published by the U. S. Department of Agriculture (68). At that meeting Paul Schreiber from Chemnitz, Germany, presented an ecological paper in which he stated:

"The growth of vegetation requires heat, water and sunshine; but of each the proper measure, as every excess or deficiency acts injuriously. It should, therefore, be the object of our investigations to determine how much of heat, water and sunshine is required by different plants, and how these influential factors are to be distributed during the various phases of plant life.

"Our information concerning the duration and power of sunshine is increasing so rapidly that we may hope for early and important additions to our knowledge concerning these elements of our investigations. If our labors in this direction are to be of practical value to the husbandman, they must include careful notations of the successive phases of plant life or, at least, of the main phases of growth—the so-called phenologic observations. If, in this manner, we discover the laws governing each separate phase or phenomenon, and from them the joint result of their reciprocal influences, our object will have been accomplished."

W. Detmer of Jena, Germany, presented a physiological paper in which he makes the following statement:

"The position of the three cardinal points, the minimum, optimum, and maximum, is by no means the same for the various physiological processes which take place in a plant or an organ; it also varies for a given process in different species of plants, and is even influenced by the degree of development of an organ."

Dr. Egon Ihne of Friedberg, Germany, made the following statement in his paper on plant phenology:

"Although the same vegetal phase may set in on a date varying from year to year, the date depending primarily on the climate of each year, yet to reach this phase the plant requires an amount of heat that is constant from year to year. A plant may, therefore, be considered as a means for measuring heat; and the beginning of a certain vegetal phase is also a standard for measuring a certain sum total of heat supplied up to that date, and this sum total expresses

the 'measure of heat required by the plant to reach the phase in question.

"Further, as has been demonstrated by physiological investigations, it is not every temperature above zero (centigrade) that is effective in vegetation, but the degree at which the temperature begins to be effective varies for different plants and phases. In the simple addition of positive temperatures, be they shade means, shade maxima, or insolation maxima, this variability finds no expression. The zero point of effective temperature should first be determined for each phase and plant in question."

Klebs (34) in 1918 emphasized the importance of recognizing the different requirements of plants during the several phases of development. In *Sempervivum Funckii* he distinguished three distinct phases, each having different environmental requirements. The same year Gassner (17) reported that earliness in winter wheat is favored when low temperatures obtain during the seedling stage and high temperatures obtain during the later stages of development.

In 1923 (48) the writer called attention to a field test in which a good crop of winter wheat was obtained in Illinois from seed sown late in November but which did not emerge until spring. In another paper McKinney and Sando (52) deal with additional literature on the subject.

From the records cited there can be no doubt that the fundamental concepts relating to vernalization and to growth-phase requirements are of long standing in America and in Europe.

METHODS

The initial steps in vernalization may be summarized as follows:

Seeds are first soaked in a suitable quantity of water to just start germination, after which they are subjected to suitable temperatures for periods ranging from about 5 to 60 days, depending on the species and variety. Or in the case of some plants as beets, cabbage, cauliflower, carrots and turnips, the young seedlings are subjected to low temperatures for suitable periods.

In general there are two ranges of temperature in use, low temperatures ranging from slightly above freezing to about 10° C. and high temperatures ranging from 20° to 30° C. It is claimed that darkness is an essential feature during exposure in the high-temperature method, but darkness is of no importance in the low-temperature treatment of germinated wheat (52) nor in the high-temperature treatment of germinated corn (64).

In all cases germination is started but retarded by holding the moisture content of the seed at suitable percentages ranging from about 45% to 60% of the dry weight of the seed, depending on the species. In some cases salts have been added to the water to prevent excessive germination during the treatment (5). At the completion of the treatment, seeds may be dried for a short time before sowing or they may be sown in a slightly moist condition.

The low-temperature treatment is used for the seeds of wheat, barley, rye, oats, timothy, rice, meadow foxtail, vetch, rape, lentils, white lupines, crimson clover, red clover, Austrian winter field peas, carrots, beets, turnips, and cabbage, and it has been applied in some modified form to such bulbs as those of the Easter lily, daffodil, Dutch and Spanish iris.

The high-temperature treatment has been advocated for corn, cotton, soybeans, millet sorghum, Sudan grass, and rice.

RESULTS

In this review it seems unnecessary to consider the results of more than a few experiments which bring out principles. In the cereal plants, emphasis will be placed on wheat and rye because these plants have been given the most intensive study and because the principles established in the studies of these plants seem to apply to other winter annual members of the Gramineae in the tribes *Hordeae* and *Aveneae*.

Cereal Plants

LOW TEMPERATURE PROCESS

In all types of wheat tested thus far by the writer and Sando (50, 52), sexual reproduction is not dependent on a critical temperature or a critical photoperiod, because this process occurs over very wide ranges of both factors. However, the time when sexual reproduction occurs and also the yield of seed are influenced by temperatures and the photoperiod (32, 33, 52) and by light intensity (52). Furthermore, the optimum conditions for the earliest sexual reproduction are not the same as the optimum conditions for the highest seed yield (5).

When winter wheats such as Harvest Queen and Turkey are grown at high temperatures in a long day throughout the life cycle, they produce many side shoots or tillers, each of which has many internodes and leaves; the elongation of the internodes, head forma-

tion, and sexual reproduction are retarded. On the other hand, when the seed is germinated slightly and chilled at temperatures slightly above freezing for 60 days before it is sown at high temperatures in a long day, the resulting plants behave as spring wheats. They develop relatively few tillers which produce few internodes and leaves, the internodes elongate rapidly and heading and sexual reproduction are accelerated. In other words, the chilling treatment has not broken a dormant period such as occurs in perennial temperate-zone fruits, but it has stimulated a directional change in the plant's activities when growth continues in a suitable environment. On the one hand, the plant vegetates excessively and sexual reproduction is delayed, whereas on the other hand vegetation is reduced and sexual reproduction is accelerated.

So far as they have been studied, winter barley (6, 7), winter oats (6, 8) and winter rye (59) have given ample evidence of reacting essentially in the same manner indicated for wheat.

Some varieties such as Purplestraw wheat are intermediate or facultative with respect to seasonal growth habit. They require a moderate amount of low temperature during early growth for early sexual reproduction. Some late spring wheats such as Kinney will head earlier if low temperatures are supplied during early growth.

In order to determine the commercial value of vernalization in the United States field trials were carried out with winter and spring wheats at Rosslyn, Va.; Lincoln and Alliance, Nebraska; Hays, Kansas; Mandan and Langdon, North Dakota; and Moccasin, Montana (51).

The most satisfactory yields were obtained at Langdon, North Dakota, the most northerly station. At that station Kanred winter wheat from seed chilled 50 days outyielded all the unchilled Marquis spring-wheat controls, though the difference was slight. Slightly greater yields were obtained from certain chilled spring wheats than from the unchilled spring-wheat controls, but the same spring varieties at Mandan gave the highest yields when the seed was not chilled.

Although rapid acceleration of sexual reproduction reduces the yield of seed per plant because of a reduction in the number of tillers and in the number of fertile florets per head (52), yields have been higher when the plants were cultured under suitable short days and low growing temperatures during the initial growth phase (50,

52) than when the germinated seeds were vernalized in the usual manner.

Harvest Queen plants from germinated seeds vernalized for 40 days near 33° F. and grown with uninterrupted light at summer temperatures, headed 89 days from the time the seed was put to soak (50). This is the most rapid heading time yet obtained in Harvest Queen winter wheat from chilled germinated seed. However, only 10 to 30 seeds per plant were produced.

Yields of 75 seeds per plant have been obtained in Harvest Queen when heading took place 88 days from the time the seed started to soak and when the seedlings and plants were grown according to the following schedule of temperatures, photoperiods, and times (50):

21.1° to 23.9° C. on moist filter paper for two days to start germination.

10.0° C. for 36 days with a photoperiod of 8 hours in culture chamber.

15.6° C. for 18 days with a photoperiod of 8 hours in culture chamber.

21.1° to 23.9° C. to end of test with a photoperiod of 18 hours in greenhouse.

These plants actually headed one day earlier than those from vernalized seed, and it is likely that still earlier heading is possible without reducing the yield to 30 seeds per plant.

Harvest Queen plants from chilled germinated seeds will yield 75 seeds if grown at 21.1° to 23.9° C. with a daily photoperiod of 16 to 18 hours. However, 100 or more days are required for plants to head when computing time from the beginning of the soaking process (50).

Although varieties of winter wheat differ in their temperature and length-of-day requirements for earliness (50), tests indicate that all the winter varieties tested complete their life cycles quite rapidly and produce good yields of seeds when grown near 7.2° to 10.0° C. with a daily photoperiod of 8 to 10 hours for 45 days, followed by temperatures near 21.1° to 23.9° C. with a daily photoperiod of 16 to 18 hours. Exposure of 20 days to the low temperatures and short days has been satisfactory for the facultative varieties such as Purplestraw.

In hybridizing work Sando and the writer have obtained essentially simultaneous flowering in early, intermediate and late varieties

of both winter and spring wheats, thus making possible all combination crosses.

In addition to hastening maturity it is claimed by Russian workers that vernalization increases drought resistance in spring cereals, and it is now claimed that drought resistance is increased when the seeds are moistened and dried intermittently three times at moderate temperatures, 20° to 22° C., allowing germination to proceed slowly during the moist stages (27, 28, 29, 30, 57, 58). Germination is started by adding water amounting to 30 per cent of the dry weight of the seed. The seed is then dried, remoistened with water amounting to 20% of the dry weight of seed, dried again, remoistened with water amounting to 15% of dry weight of seed, dried and sown. The object is to allow very slight progress of germination at each moistening and to keep the plumules and roots from developing to a point which prevents easy drilling. The method is referred to as prehardening.

Data cited by Zuhr (77) indicate that vernalization reduced bunt and loose smut in a spring variety and in a facultative variety of wheat grown in the field. Loose smut was reduced 45.3% to 68.3% in comparison with non-vernalized controls.

These aspects of the vernalization problem have not been tested to any extent in other parts of the world.

Vasiljev (69), Timofeeva (66) and others found that winter hardiness in winter wheats is reduced by vernalization. It appears that vernalization of the germinated seed before sowing prevents the normal hardening which takes place in the field during the autumn and early winter.

Buřlina (9) found that the optimum time for vernalization tends to increase with the degree of winter hardiness characteristic of a given variety of winter wheat. The varieties having low winter-hardiness required 45 to 55 days, those of medium hardiness required 55 to 60 days, whereas the most winterhardy required 60 to 65 days.

Rice was vernalized at 3° to 5° C. by Ossewaarde (55) in Holland. The time periods were 2 weeks and 5 weeks. The vegetative period was shortened 2 to 7 days and the yield of straw and grain were increased by the two-weeks treatment.

The high-temperature method has been used also for vernalizing rice as indicated below.

HIGH-TEMPERATURE PROCESS

It has been claimed that certain summer annuals have a short vegetative period when the slightly germinated seeds are held for 5 to 15 days at temperature ranging from 20° to 30° C. in darkness (45).

Seed of several cereal crop plants has been treated according to the methods recommended and comparisons have been made in the field between the plants from the treated and the nontreated seed.

Sprague (64) working with several hybrids, inbreds and one variety of corn *Zea mays* gave the method a rather thorough trial at the Arlington Experiment Farm.

Although the seed was soaked in a 0.5% solution of Uspulun to cut down molds, vernalization reduced the germination greatly over the controls and caused delay in emergence in most cases.

Vernalization reduced the number of internodes very slightly and hastened sexual maturity. However, the differences in time of maturity between the vernalized and the control series were so slight as to be of no agronomic importance. Yields of grain were reduced in the vernalized series. In the five comparisons which were significant the reduction was approximately 15% to 30%. As in the case of wheat the day length following vernalization influences the time of maturity of the corn plant. Sixteen hours of light daily during culture hastened sexual maturity from 10 to 15 days over continuous light. Vernalization was just as effective in continuous light as it was in the dark.

Tests with vernalized corn were conducted by Elema (15) in Holland and the results agree in general with those reported by Sprague.

Martin reports (53) that seed of 41 varieties of sorghum were vernalized by the recommended high-temperature method and all varieties failed to mature earlier than the controls. In most varieties vernalization greatly reduced or completely destroyed germination. Similar results have been reported from Holland by Elema (15) and van Hoek (31).

Haigh conducted tests with rice in Ceylon, and his results were reported in a personal communication to the Imperial Bureau of Plant Genetics (2). Seed was soaked for 24 hours, then held for 6 days at three different temperatures, 25°, 30°, and 35° C. Sowings were made in the field. Vernalization favored flowering by

5 to 10 days in comparison with the dry-seed controls, and by 1 to 4 days in comparison with the controls sown with germinated, non-vernalized seed. The method has no practical value, according to the report.

Forage Plants

Čepikova (11, 12) reports that *Alopecurus pratensis* (meadow foxtail) and *Phleum pratense* (timothy) produced seed the first season when the germinated seeds were first vernalized near 3° C. *Trifolium pratense* (red clover, double-cut type) produced seed the first season when the seed was vernalized at 10° to 12° C. for 10 days.

Zerling and Čepikova (75, 76) carried out additional tests and found that *Trifolium pratense* (red clover, single-cut type) seeded the first season when vernalized 20 to 40 days at 3° to 8° C. These investigators report that vernalized meadow foxtail, timothy and red clover were earlier and gave higher yields than the controls the second season. In other words, the autumn, winter and spring conditions seemingly did not have an equalizing effect on the controls when compared with the vernalized lots. The timothy seemed to show the greatest benefit the second season from vernalization of the year previous.

Kostov (38) reported favorable results with vetch vernalized for 10 days at 8° to 10° C.

Žvanskiĭ (73) and Žvanskiĭ and Spilevskii (74) report that seed was obtained from winter rape the first season when the germinated seeds were kept in snow for 16 days and in a cool cellar for 15 days before sowing on April 23.

Soybean seeds were vernalized by the high-temperature method in Holland by Elema (15) and by van Hoek (31) and in England at the Long Ashton Station (2, 118). All reports indicate that plants from vernalized seed behaved as the plants from the non-vernalized seed. Negative results were reported from Long Ashton with respect to peas vernalized by the low-temperature method.

In the United States McKee (47) tested the effect of low-temperature vernalization on White lupine (*Lupinus albus*), crimson clover (*Trifolium incarnatum*), hairy vetch (*Vicia villosa*), Austrian Winter field pea (*Pisum arvense*), double-cut red clover (*T. pratense*), and white sweet clover (*Melilotus alba*). Germinated

seeds were held at 0° C. for 40 days before sowing. All species except the red clover and sweet clover came into flower and fruit when vernalized. Nonvernalized lots either did not bloom or were late. The temperatures were probably too low and the time too long in the case of the clovers.

The same investigator tested foxtail millet (*Chaetachloa italica*), Sudan grass (*Sorghum vulgare sudanense*), soybean (*Soja max*) and crotalaria by the high-temperature method of vernalization. In most cases vernalization decreased vigor and in no case did the treatment hasten the time of maturity.

Miscellaneous Plants

Burr and Turner (10) vernalized seeds of tomato and cucumber at 1° to 3° C. for 7 to 44 days before sowing. Vernalization reduced germination, stunted the plants and reduced fruit yields. In later tests these investigators (Turner and Burr 67) found that vernalization at -0.28° C. followed by continuous illumination of the seedling and young plants for 2 to 24 days hastened maturity and increased the yield of fruit in tomato. The failure of the first test is attributed to the fact that the vernalized seed was kept too long before planting.

Yamamoto (72) reports that seed production is favored in radish (*Raphanus sativus* L.) when the small seedlings are first subjected to 0° to 5° C. for 10 to 15 days before placing at warm temperatures.

Van Hoek (31) vernalized potato tubers in a lighted box at 18° to 20° C. for 26 days. Plants from vernalized tubers grew more rapidly, were more vigorous and matured 6 days sooner than the controls. Twenty-three hills from vernalized and from nonvernalized tubers yielded 22.3 and 20.6 kg tubers, respectively. Tubers from the vernalized series were larger than those from the control.

Chesnokov (13) tested beet seeds (*Beta vulgaris* var. Egyptian and Bordeaux) vernalized 43 days and 68 days at 3° to 5° C. The longer time of exposure increased the amount of bolting the first season. This treatment induced bolting in almost 2/3 of the plants in both varieties and seed was produced. Similar results have been reported by others (4).

In southern New Mexico (56), high yields of sugar beet seed are obtained in late June and July from seed sown in the field dur-

ing late August or early September. Here vernalization takes place naturally during the mild winter.

In a later paper Chesnokov (14) states that 80% to 90% of the beet plants bolted the first season when the young seedlings were chilled for 50 days. He claims turnip, cabbage and carrot seeded more freely the first season when the young seedlings were chilled than when slightly germinated seeds were chilled.

Taşlanov and Pudovkina (65) vernalized germinated cotton seeds at 13° to 23° C. for 3 days followed by 25° to 30° C. for 13 days. Vernalization hastened flowering and maturity 9 to 11 days in the Egyptian varieties; acceleration was especially evident in the late varieties. The American varieties showed less acceleration and more variations between themselves than was the case in the Egyptian types. It is claimed that the yields of all Egyptian varieties and part of the American varieties were increased. Tests carried out with cotton in Indore (2, 134) led to the conclusion that vernalization has no commercial advantage in that locality.

The acceleration of blooming in ornamental plants has been practiced for many years by growers and by amateurs. A recent book by Lawrie and Poech (40) covers this field rather fully. Some of the methods used involve the breaking of a true rest period previous to the final treatments which accelerate blooming. High and low temperatures during storage and after potting or planting are used.

The acceleration of blooming in daffodil and bulbous iris was studied by Griffiths (25). He found that daffodil bulbs should be kept under ordinary shed storage conditions. If temperatures go below 21° C. additional heat should be supplied. When the buds are visible—about August 1—they are stored at 10° C. for about one month, then potted and held at 10° to 18° C. for about a month in a cellar. If the bulbs are then benched in a greenhouse with night temperatures gradually increasing from 10° to 15° C., flowering begins just before Christmas, depending on the variety.

Early blooming in Dutch and Spanish iris is accomplished by storing at 26.7° C. from the time of digging to August 1. After this treatment they are stored at 10° C. for a month, then planted in a well ventilated greenhouse. Blooming occurs in December and January, depending on the variety.

In Bermuda (3) growers and experimenters accelerate blooming in the Easter lily when bulbs are stored at 2.22° C. for a month before planting October 15.

MORPHOLOGICAL AND PHYSIOLOGICAL STUDIES

McKinney and Sando (50) reported that earliness in wheat is correlated rather closely with a number of leaves and internodes produced by the culms or stalks. A number of early, intermediate and late spring varieties were studied with different daily photoperiods in two ranges of temperature. Early varieties have fewer leaves and internodes than late ones, also temperature and light conditions favoring few leaves and internodes in a given variety tend to favor earliness except that change in time of flowering is somewhat more sensitive to environmental change than is the internode number. For example, Prelude headed from 32 to 43 days after planting with a leaf number of 5. When conditions induced 11 leaves per culm, heading was extended to 140 days. The variety Reward headed from 33 to 53 days from planting with a leaf number of 6. When 13 leaves per culm were produced, heading was extended to 104 days.

Under conditions favoring the earliest heading—continuous light at summer temperatures—the earliest varieties produced 5 leaves and the latest produced 11 leaves per culm.

Purvis (59) working with winter rye found that earliness is favored by the conditions which favor few leaves per culm. The same results were also observed and recorded by McKinney and Sando (52) for winter wheat. Using Petkus spring and winter rye Purvis and Gregory (60) found that approximately the first seven of the lateral primordia of the main axis are obligate leaf primordia, the subsequent 18 lateral primordia are labile or facultative in that they may become leaves or spikelets, depending on the temperature and photoperiod, and the subsequent ones are obligate spikelet primordia.

Harvest Queen winter wheat (52) has approximately seven obligate leaf primordia and approximately fifteen labile primordia. These numbers are inherently less in the early spring and winter wheats and greater in the late varieties.

The number of stalks per plant (tillering) (6, 16, 32, 59) and the number of seeds per plant are intimately connected with earliness. The writer and Sando (50) reported that few tillers and small numbers of seeds result when winter wheat is forced to complete its life cycle too rapidly, but that seed yields are higher when forcing is less rapid and is accomplished by means of low growing

temperatures with short days, followed by the higher temperatures and long days, than is the case when the germinated seeds are vernalized before seeding and the subsequent plants are forced by high temperatures and long days.

The writer with Sando (52) found that immature germinated seeds of Harvest Queen winter wheat vernalized as efficiently as mature germinated seeds in a refrigerator. However, they did not test seeds earlier than the soft-dough stage. Kostjucenko and Zarubailo (36) report that the maturing seeds of winter wheat are vernalized naturally in the field in northern areas when the temperatures are sufficiently low before the seed is mature. Gregory and Purvis (19, 22) report the same phenomenon in winter rye but their tests with wheat failed. They found that winter-rye heads chilled during the middle period of ripening vernalized, whereas those chilled before and after this period did not. They (18, 22, 24) also succeeded in vernalizing excised germinated winter-rye embryos on nutrient agar containing carbohydrate at 1° C. In later tests Gregory and de Ropp (24) found excised embryos grown on nutrient agar containing no carbohydrate failed to vernalize whereas those on agar containing 3% sucrose vernalized.

Kostjucenko and Zarubailo (37) in summarizing their work conclude that the milk-ripe stage responds to vernalization because the physiology of the seed at this time is nearly comparable to the seed during germination. They cite data relating to the carbohydrate content, peroxidase and catalase activities in support of their conclusion. These investigators conducted field tests in northern and more southern areas in Russia and found that natural vernalization during maturity in the north shortened the vegetative period the following season when the seed was sown at more southern stations in comparison with plants from seed grown at the southern station the previous season. They point out that natural vernalization must be taken into account when seed of winter and late spring cereals is taken from northern to southern areas, otherwise genetic and agronomic results may not be properly interpreted. They indicate that winter hardiness is greatly reduced by natural vernalization and they recommend that seed of winter cereals intended for fall sowing in north Russia come from the more southern regions where natural vernalization does not occur. Temperatures at 15° C. and below during the milk stage of the seed are regarded as favoring natural vernalization.

In the United States, the daily mean temperature may reach 15° C. during the midperiod of seed development at some points near the Canadian border. However, practically no winter wheat is grown in that latitude and it is still a question as to the vernalization response in the Durum wheats which are later than most of the common spring varieties.

In their studies on winter rye Gregory and Purvis (20) found that devernalization can take place. They ran a test in 5 parts, each for a period of 6 weeks. Germinated seeds were subjected to 1° C. and to 20° C. in darkness for alternating periods. The 1° C. treatments were for 1, 2, and 3 and 6 day intervals, respectively. After each of these intervals there was in each case exposure to 20° C. for 1 day, followed by the respective schedules above at 1° C. While at 1° C. the seedlings had access to the ordinary or normal atmosphere but while at 20° C. the seeds were in an atmosphere of nitrogen. A control received 1° C. every day, but the nitrogen and ordinary atmosphere were alternated daily. After the treatments the seeds were planted and cultured at suitable growing temperatures and photoperiods. The seed lots receiving the 1-degree treatments for 1, 2, 3 and 6 consecutive days and the control seed lot receiving 1° C. continuously had 0, 20, 60, 100 and 100 per cent of the seeds vernalized, respectively. It is concluded that high temperature nullifies the effect of low temperature.

In later tests these investigators also (21, 23) found that an atmosphere of nitrogen extended the vegetative period in spring rye. Rye seeds thus devernalized were reveralized when subjected to 1° C. for 3 weeks in the normal atmosphere.

Winter rye (22) which had been vernalized and then devernalized lost its ability to head early at high temperatures, but it produced more tillers at high temperatures than rye which had never been vernalized. ✓

From the results cited it is to be expected that warm day temperatures will nullify the natural vernalization induced in maturing seeds by low night temperatures until a sufficiently low daily mean temperature is reached. The exact relative efficiencies of low temperatures for vernalization and of high temperatures for devernalization when these phenomena are working against each other apparently has not been determined in terms of time and degrees of temperature.

Germination, though progressed very slightly, and at least 50% moisture are essential for the successful vernalization of mature seeds (41, 43, 44, 52), when the temperature is at optimum.

Darkness and light had no apparent influence on the vernalization efficiency of low temperatures in the case of germinated winter-wheat seed (52).

Turkey winter wheat seed was held at 3.3° C. for 61 days. One sample was in total darkness during the test and another sample received daylight during the entire day each day of the test. After vernalization the seeds were sown outdoors during early summer with the natural photoperiod. The plants from seed chilled in daylight headed 47 days after sowing whereas the plants from seed chilled in darkness headed 49 days after sowing. A difference of two days is not significant thus indicating that low temperatures and not darkness stimulated the early heading. Turkey wheat sown during the summer without vernalization does not head in the vicinity of Washington, D. C.

Whyte (70) indicates that these findings are inconsistent with earlier findings published by McKinney and Sando (49, 50, 52). However, the writer fails to find such inconsistency. The earlier tests referred to relate to plants growing at temperatures considerably above 38° F. and in short days vs. long days, whereas the light and dark test referred to above was carried out at 3.3° C. with germinated seeds, essentially the conditions of seed vernalization, and the conclusion relates only to slightly germinated seed (vernalization), a point which seems clear enough from the chapter headings and descriptions in that paper (52, 630).

Tests and observations reported by the writer and Sando (52) ruled out the endosperm, the tips of the roots, coleoptile tip and tip of the first true leaf of seedlings as the sole active centers of sensitivity to the vernalization process.

Krasnoseljskaja-Maximova (39) claimed that spring cereals contain no detectable substance which favors early flowering but that the winter cereals contain an inhibiting substance which must be counteracted before the plants can proceed to sexual reproduction. Later Sereiskii and Sluckaja (62) claimed that winter wheat contains no such inhibitor.

Richter (61) in his report as director summarizes the results obtained in studies conducted under the direction of Cailahjan on vernalization and photoperiodism as follows:

Vernalization shifted the iso-electric point of albumino-lipoids towards the acid end, increased the permeability of the protoplasm and the mobility of the albuminous complex, intensified photosynthesis, increased dry matter in insoluble proteins, and decreased soluble protein. It is claimed that sexual processes controlled by light occur in the leaves and are related to the formation of flower hormones (floregin) which moves to the promeristem. In grafting experiments the floregin was transferred from stock to scion. Floregin was found not to be specific for species or biologic forms. It is claimed that no substance inhibiting or retarding flowering is formed in the leaves.

Purvis and Gregory (22) have shown from studies with excised embryos and with ripening grain that vernalization in winter rye is localized in the embryo and is independent of changes in the endosperm or aleurone layer. They conclude that the growing embryo is able to synthesize hormones at low temperatures from a substrate containing glucose, and inorganic salts including nitrates. It is their idea that a precursor (*A*) in the embryo of winter rye is converted by autocatalysis at low temperatures into a substance (*B*) which in turn may be converted into a spikelet initiating substance (*C*) and a spikelet maturation substance (*D*) or into a vegetative leaf-promoting substance (*E*), depending on the subsequent photoperiod or temperature, and the system $C \rightleftharpoons B$ is reversible. Devernalization due to drying is accounted for by a conversion of substance (*B*) to substance (*E*). Substance (*B*) is naturally in high concentration in spring varieties.

DISCUSSION

In general the chilling method of vernalization has been found to accelerate sexual reproduction with greater certainty than the high-temperature method in the particular species for which each method has been recommended. Many workers report that they have been unable to obtain acceleration with the high-temperature method and in those cases where acceleration has occurred the commercial advantage has not been evident.

For several years vernalization methods have been tested in many parts of the world and it is noteworthy that the majority of investigators outside of Russia fail to recognize any great commercial value to be derived from the methods as applied to the small grains, rice, corn, sorghum, forage crops and cotton in the regions where

these crops are adapted. It seems to be the general consensus of opinion that the crop problems can best be solved through developing better adapted genotypes.

Some commercial value is attached to the chilling method when used to force flowering in daffodils, Dutch and Spanish iris, and Easter lily. The method seems to offer commercial possibilities for speeding up seed production in certain biennials such as the garden beet and sugar beet, and it has considerable value in speeding up seed production in genetic and plant-improvement work with many crop plants. However, entirely aside from the stimulating influence of low temperatures during germination, it is strikingly evident that the temperature, the photoperiod, the intensity and the quality of light during the entire period of growth have marked influence on the time when sexual reproduction occurs and it is only when these factors are understood in relation to all the growth phases of the particular genotype that the most satisfactory results can be obtained from the initial chilling.

In view of the evidence, it seems justifiable to conclude that Harvest Queen, Turkey and similar winter wheats and other winter cereals are not typical long-day plants with respect to their earliest sexual reproduction, but are what may be termed short-day \rightarrow long-day plants, and they may be considered as low-temperature \rightarrow high-temperature plants. This method of expression indicates that low temperature or short days and low temperatures in combination must obtain in the germinating seeds or in the young growing plants, respectively, for suitable periods in order that the first labile primordium and subsequent ones will develop into spikelets instead of leaves when the high temperatures and the long days are introduced. A similar situation seems to apply to the facultative varieties and to certain late varieties commonly placed in the spring group, but in these the initial optimum temperatures are either not so low or the periods of exposure to low temperatures are shorter than is the case with the strictly winter varieties.

The typical spring cereals are high-temperature and long-day plants in the truest sense with respect to early sexual reproduction. However, as in the case of winter varieties the spring varieties differ with respect to their optimal environmental requirements for the earliest completion of the life cycle.

Earliness of sexual reproduction appears to depend on the interrelation of several plant characters, *i.e.*, (1) characteristic number

of obligate leaf primordia and attending internodes, (2) characteristics of the embryo which bring into existence or activate substances which in turn activate the labile lateral primordia into spikelets at characteristic (*a*) temperatures, (*b*) photoperiods and (*c*) periods of time, (3) growth characteristics of the stem internodes and (4) rate of maturity of the sex organs and seeds.

Conditions favoring the earliest sexual reproduction induce relatively low yields of seed per plant. In Marquis spring wheat and Harvest Queen winter wheat high seed yields obtain when five to seven of the labile primordia produce leaves and when the heads on 3 to 4 stalks have 14 to 15 well-filled spikelets. This is accomplished by gradual changes from the lower to the higher growing temperatures and from the short to the longer days. The total time requirement is greater than required for the earliest reproduction.

On the basis of the reversals in the order of heading in certain pairs of varieties when grown in different environments, it can be concluded that segregating populations from certain parent crosses will not give constant segregating ratios for earliness under all conditions of temperature and day length. As pointed out in a previous paper (50), populations which are segregating for earliness and lateness should be tested and classified as far as practicable under several conditions of temperature and photoperiod to facilitate the selection of genotypes homozygous for the several characters influencing earliness and lateness.

These conclusions do not vitiate the fundamental postulates of evolution or of genetics but they do indicate that a knowledge of growth phases and character expression in relation to environmental factors will facilitate the adequate planning of many genetic studies and the interpretation of the results. Such characters as yield, seasonal growth habit, recumbence, relative earliness, resistance and susceptibility to certain parasites, to extreme temperatures and to drought, serve to illustrate a few of the complexes which can be studied profitably under several conditions of environment for the purpose of determining at least some of the simpler characters of which they are composed. In critical genetic studies these simpler characters offer advantages over the complex ones.

The responses of the healthy or the diseased plant to temperature, the photoperiod, light intensity, light quality, humidity, soil moisture, soil fertility, etc. are most certainly determined by internal mechanisms which in turn constitute genetic characters, and

although these characters cannot be measured directly at present, in some cases they can be measured indirectly and be dealt with as characters. As this procedure is followed, and as it becomes more generally recognized that dominance and segregation ratios in many characters must be considered within well defined environmental limits, some of the confusion regarding the environment and inheritance will disappear.

The various experimental results discussed in this paper seem to shed no direct light on Lysenko's¹ view that certain genotypes can be altered by systematic culture in an environment which differs from that naturally required by the plant. This so-called alteration of the genotype—referred to as “training” the plant—would virtually amount to directed mutation on a mass-production scale. To settle this point it would be necessary to plan and conduct the experiments in a somewhat different manner than was followed by the writer and the other investigators cited in the vernalization and growth-phase studies.

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THE PROBLEM OF GOLGI MATERIAL IN PLANT CELLS

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The name "Golgi apparatus" was first applied by investigators to the net-like "apparato reticolare interno" described by Golgi (1898) in nerve cells of the barn owl fixed by the formalin-arsenious acid-silver nitrate method. Since Golgi's time a variety of new and different fixation methods involving usually silver nitrate or osmium tetroxide (osmic acid) have been developed, and many structures believed to be homologous with the original Golgi apparatus have been discovered and described. However, because these structures were not always reticular, a number of new terms have been applied to bodies which in the aggregate were believed to constitute the Golgi apparatus of the cell. In the interest of clarification in this review, it seems desirable to list some of the terms used as synonyms of all or a part of the Golgi apparatus. These are Golgi elements, Golgi bodies, Golgi net, Golgi crescents, Golgi cups, Golgi vesicles, Golgi globules, Golgi vacuoles, Golgi granules, Golgi filaments, dictyosomes, batonettes, vacuome, osmiophilic platelets, trophospongia, Holmgren's canals, Nebenkern, Golgi complex, lacunae, lepidosomes, and para-golgien material. Gatenby (1930) included a glossary of some of these terms in his discussion of cell nomenclature. Furthermore, many writers refer to "osmiophilic" or chromophilic, and "osmiophobic" or chromophobic portions of the Golgi bodies. The more lightly stained osmiophobic substance has been called the "archoplasm" or "sphere substance," and believed to be chemically different from the osmiophilic cortex. However, according to Hoerr (1936), these differences in intensity of blackening are more probably due to differences in the penetration of the fixatives.

*I wish to express my appreciation to The New York Botanical Garden for permitting me to use its Library facilities in the preparation of this review.

The purpose of the present review is to summarize the history and present status of the Golgi bodies of plant cells. Reference to work on animal cells is made only when necessary to clarify a viewpoint or present a technique. The review of Kirkman and Sevringhaus (1938) includes an excellent bibliography on Golgi material in animal cells. The conclusion of these two authors as to the present status of the Golgi question is quite different from that gleaned by a survey of the recent literature pertaining to the Golgi material of plant cells.

MORPHOLOGY AND TERMINOLOGY

Although the Golgi apparatus had been identified before 1920 in various types of animal cells, the majority of the descriptions of Golgi bodies in plant cells were made later. Drew (1920) observed darkly staining oval or elongated bodies in cells of root tips of the onion and suggested that they were homologous with the Golgi apparatus of animal cells. Drew did not use osmic or silver impregnation methods, and there is some doubt as to the exact homology of the structures he described.

In the decade following Drew's work two principal theories concerning the plant Golgi bodies were developed. One school, represented by Patten, Scott and Gatenby, Bowen, and Beams and King, described in many types of plant cells structures known as osmophilic platelets. Each platelet was a blackened plaque with a peripheral rim of specifically different composition and, depending upon the plane of observation, appeared as a rim, a rod, or an ellipse. The second school, represented particularly by Mangenot and Guilliermond, held that the plant vacuolar system or "vacuome," as it had been named by Dangeard (1916), was homologous with the Golgi apparatus of animal cells. These two opposing points of view are summarized by Zirkle (1937), but will be discussed here in somewhat greater detail.

Bowen (1927*a* and 1928*a*), after studying a great many types of animal cells, began researches on the Golgi apparatus of plant cells. He examined osmicated preparations of representative bryophytes (male heads of *Polytrichum*), pteridophytes (growing point and root tip of *Equisetum arvense*), and spermatophytes (young shoots of *Hordeum vulgare* and root tips of *Vicia*, *Pisum*, *Ricinus*, *Hyalcinthus*, *Hordeum*, *Cucurbita pepo*, and *Phaseolus*), and recorded a "surprising uniformity" in the morphological components of the

cytoplasm of plant cells. Osmiophilic platelets were the most generally demonstrated formed component, but plastidome and pseudochondriome were also described. The osmiophilic platelets were believed to be the Golgi bodies, and Bowen considered the negligence of botanists in not having studied them sooner "truly regrettable." Bowen and Buck (1930) extended their investigations to include gymnosperms (entire young seedlings, growing ends, and root tips of *Pinus mughus* and *P. austriaca*), and the results confirmed those obtained with the other groups of plants.

Patten, Scott, and Gatenby (1928) made a study of young root tips of *Vicia faba* and *Hyacinthus* and shoots of *Pisum*, and found themselves in essential agreement with Bowen. They reiterated the theory that the osmiophilic platelets were distinct morphological entities of plant cells and constituted the Golgi apparatus of plant cells.

Beams and King (1935) ultra-centrifuged root tips of *Phaseolus vulgaris*, fixed them by osmic acid methods, and described them according to Bowen's terminology. Although they stated that this terminology was not entirely satisfactory, they failed to state in what particular respects it was inadequate. The vacuome, when present, was found to be the cellular constituent of lowest specific gravity. Osmiophilic platelets were next in order, and the pseudochondriome was heavier than either. Beams and King, therefore, supported Bowen's contention that osmiophilic platelets were distinct cellular entities and the homologue of the animal Golgi apparatus.

The second of the two principal theories regarding plant Golgi bodies was suggested somewhat earlier. In fact, the homology of the plant cell vacuole with the Golgi apparatus and Holmgren's canals of animal cells was inferred by Bensley (1910). After giving a rather complete history of research on Golgi bodies prior to 1910, Bensley reported his own investigations on root tip cells of *Allium*, *Lilium*, and *Iris*. He believed the plant vacuolar system to be canal-like from its earliest inception in meristem cells and, therefore, homologous with Holmgren's canals of animal cells which, in turn, had previously been homologized with the Golgi apparatus.

Bensley's conception of the Golgi apparatus of plant cells was revived by Guilliermond and Mangelot (1922) who believed that the vacuome corresponded to both the Golgi apparatus and Holm-

gren's canals. The Golgi apparatus was the positive picture produced by precipitation of silver nitrate within the vacuoles, and Holmgren's canals the negative image resulting when the vacuolar content was not fixed. Guilliermond continued his work and extended his conclusions in 1929. The osmiophilic platelets were believed to be the result of transformation of some other cellular component during the fixation process.

Guilliermond's work was substantiated by that of Parat (1928) who found a system of vacuoles in animal cells after staining with neutral red. Parat identified the dictyosomes or "classical Golgi" bodies of other investigators as the active chondriosomes or "lepidosomes." Neither Parat nor Guilliermond was able to demonstrate a reticular Golgi apparatus which could not be identified as a modification resulting from the fixation of the vacuome. Parat believed that the Golgi nets figured by other investigators were artifacts produced by coalescence of the vacuoles around which osmic acid was reduced by the aggregated lipoids of the vacuoles and the lepidosomes. The name "Golgi apparatus" was therefore transferred from the reticular apparatus, which had originally borne it, to the vacuome. Such transfer of terminology can scarcely be defended.

Several investigators have confirmed Guilliermond's results. Scott (1929) observed the canal-like vacuome in all stages of development in root cells of *Vicia faba*, and concluded that it made no particular difference whether it was called "Golgi apparatus" or "vacuome," the terminology depending upon the appearance of the section following osmication. She believed, however, that the osmic-acid reducing substances were somewhat transitory within the vacuome. Bose (1931) used small pieces of hymenial surface tissue of twelve species of fungi (common polypores and agarics of India), and the only blackened nets he was able to identify resulted from excessive precipitation of silver and osmium within the vacuome. The vacuome likewise was the only structure which stained with neutral red. Cassaigne (1931) observed that the vacuoles of *Saprolegnia* and yeast occasionally formed nets. Gonçalves da Cunha (1932) noted variations in the form of the vacuome in cells of developing grains of wheat after staining with silver nitrate. The vacuome sometimes became a typical irregular Golgi network and on other occasions consisted of blackened spherical bodies. He be-

lieved the difference to be due to the consistency of the vacuolar content. If it were fluid or solid the vacuoles remained separate, but if semi-fluid they coalesced to form the reticulum of fixed preparations.

Bowen (1927*b*) compiled a table comparing his conception of the structure of plant and animal cells with that of Guilliermond and of the Dangeards (1916 and 1923), and pointed out that the Dangeards had not mentioned the Golgi apparatus, but that Guilliermond had suggested the homology. Dangeard (1930) summarized his conception of cellular structure and ranked the vacuome second only to the nucleus in importance as a cell constituent, but made no direct statement concerning homology of the vacuome and the Golgi apparatus.

The vacuome hypothesis has met much criticism. Bowen (1928*a*) found himself unable to accept it. Although his procedures had resulted occasionally in a blackening of the vacuome, he attributed this to the presence of a lipid vacuolar membrane, and to the occasional fusion of small vacuoles to form a net-like structure not homologous with the "true" Golgi bodies, the osmiophilic platelets. Gatenby (1930) criticized the proponents of the vacuome theory and welcomed the discovery of osmiophilic platelets as refreshing after the "confusion" of the French school. Chang (1935) pointed out that Parat had failed to demonstrate the homology of the neutral red-stained bodies of animal cells and the vacuome of plant cells before he applied the term vacuome to animal cells. Certainly Parat's work did little to simplify the problem. Weier (1932*b*), without accepting the osmiophilic platelet hypothesis, listed eight reasons for his inability to accept the vacuome theory. Probably the most significant of these is involved in an argument about Golgi function: "in the animal cell the osmiophilic substance plays a part in the elaboration of the secretory product, in the plant cell the osmiophilic vacuome is the secretory product."

The osmiophilic platelet hypothesis has likewise been criticized. Bose (1931) contended that Bowen's osmiophilic platelets were nothing but distorted mitosomes or the "petit batonnettes" of Guilliermond. Py (1932) found that the vacuome in tapetal cells of plants of fifteen genera was remarkably constant in form, but that plastids often had impregnated margins which caused them to resemble osmiophilic platelets. Kiyohara (1930) observed osmiophilic platelets in cells of *Hydrilla verticillata*, *Elodea canadensis*,

and *Pilea viridissima*, but was able to demonstrate a similar appearance of the plastids of potato tubers after Carnoy's fixation. He concluded on the basis of this evidence that the osmiophilic platelets were nothing more than young amyloplasts.

In addition to the two principal theories, there are a number of other minor ones.

Weier (1931, 1932a, b) suggested that the plastids fulfilled the requirements for a Golgi apparatus. The plastids of *Polytrichum*, *Anthoceros*, *Zea mays*, and *Elodea* were observed to consist of a chromophilic portion, the "plastonema," surrounding the grayish "plastosome." Weier next compared his slides with Golgi preparations of animal tissues made earlier by Bowen and by Beams. He was able to find many examples of close correspondence between the appearance of the Golgi apparatus in the different types of animal tissues, and the varying forms of the plastids of different types of plant cells. Although Weier did not actually state that he believed the plastid to be the Golgi apparatus, he did emphasize the similarities of the two structures. The entire hypothesis seems a trifle far fetched, and it is of interest to note that in recent publications (1938b) Weier did not mention the Golgi apparatus.

Salazar (1936) described a "Golgi complex" composed essentially of a Golgi apparatus interspersed with granules of "paragolgien" material in animal cells. He believed differences in the morphology of the Golgi net represented merely cellular stages of activity and age.

Sass (1934) used no Golgi techniques but believed a large hyaline body, the Nebenkern, which he identified in the pileus of *Coprinus sterquilinus*, to be the Golgi apparatus. The homology was based on a resemblance of the Nebenkern to the Golgi apparatus observed during spermatogenesis in certain animals. The evidence advanced to support Sass' theory seems definitely inadequate.

Chadefaud (1930) noted numerous small bodies which stained with neutral red in cells of *Conferva bombycina*. The structures were distinct from the vacuome and were named "physodes." Chadefaud believed them to be homologous with Parat's neutral red vacuoles of animal cells.

Hovasse (1936, 1939) described dictyosomes surrounding the nucleus in cells of Volvocales. Each dictyosome was composed of outer chromophilic and inner chromophobic areas. Chadefaud

(1937) observed similar short, thick, and occasionally vesicular dictyosomes closely applied to the nucleus of fixed cells of *Ulothrix* and *Microspora*. These bodies were readily visible in living cells, insoluble in fat solvents, and lacked any affinity for the vital stains used to demonstrate the vacuome. The position of the dictyosomes described by Hovasse and Chadeaud is approximately that of the chromosomes in many resting nuclei.

The question of what, if anything, constitutes the Golgi apparatus of unicellular organisms has also been much debated. Hall (1936) reviewed the literature pertaining to the cytoplasmic inclusions of the Phytomastigoda. He concluded that in this group, as in plants in general, the Golgi apparatus was composed of a vacuome which stained vitally with neutral red and was impregnated by the silver and osmic Golgi techniques. Hall stated that the vacuome, stigma, contractile vacuoles, and specialized organelles had been identified as Golgi bodies at one time or another. To illustrate these different points of view, four investigations other than those of Hall on flagellates may be cited. Chadeaud (1938) described the structure of a new variety of *Euglena mutabilis*, but did not observe Golgi bodies or dictyosomes. Baker (1933), using *Euglena gracilis*, observed both neutral red vacuoles and a number of small blackened "Golgi globules." Each of the Golgi globules possessed a chromophilic outer layer and a chromophobic center, and could be distinguished from the vacuome by its ability to resist bleaching. Duboscq and Grasse (1933) considered the parabasal apparatus of flagellates Golgi material. Gatenby and Singh (1938) and Gatenby, Singh, and Browne (1938) described still another type of Golgi material in a *gracilis*-like species of *Euglena* and in *Copromonas subtilis*. In *Euglena* numerous loaf-shaped Golgi elements were closely applied to the contractile vacuole. The osmiophile material in some specimens of *Copromonas* was comparable with that of *Euglena*, but in others it formed a thick cortex about the structure known as the reservoir. In both species the Golgi material persisted throughout division and encystment and was capable of perpetuating itself by dictyokinesis (division). However, in *Copromonas* the osmiophile material occasionally left the reservoir during cell division and "floated down" nearer the center of the cell. Gatenby, Singh, and Browne interpreted these facts as indicative of the evolutionary origin of the Golgi apparatus among primitive flagellates where it was associated with the base of the flagellum.

It later became associated with the contractile vacuole as in *Euglena*. *Copromonas* represented an intermediate condition because its Golgi material sometimes migrated during cell division.

A few investigators have been unable to find any constant cellular unit which they were willing to accept as the Golgi apparatus. Nevins (1933), studying cells of *Sphaerocarpos* during spermatogenesis, was able to identify osmiophilic platelets in only a very small number of the cells. The platelets were sometimes present in young androcytes, but were absent from the young antheridia which produced the androcytes and disappeared again as the metamorphosis of the androcyte proceeded. She interpreted stages which Bowen believed to indicate division of Golgi material as optical illusions. Nevins also studied nectar-secreting parts of flowers of *Impatiens*, *Delphinium*, *Abutilon Darwinii*, and lemon, but was unable to identify any Golgi bodies because all tissues were blackened intensely and bleaching with turpentine failed to differentiate them. Although Bowen had not found vacuoles in androgonial cells of *Polytrichum*, Nevins saw large vacuoles in corresponding tissues of *Sphaerocarpos*. The vacuoles, however, seldom contained stainable material and did not resemble the reticular apparatus shown by Guilliermond. In a few preparations both osmiophilic platelets and vacuoles were demonstrated simultaneously and thus were shown to be entirely independent.

Guilliermond (1935) modified his views, decided that the argument concerning the Golgi apparatus was fruitless, and advocated the abandonment of the term as a name for any part of the plant cell. He based his suggestion on the fact that in all his investigations he had found no structures other than the vacuome and the chondriosomes which could be homologized with Golgi bodies. Because these structures already possessed names, the term "Golgi apparatus" was superfluous. Guilliermond described the transformation of chondriosomes into dictyosomes or osmiophilic platelets during fixation. He observed that the mitochondria (spherical forms) became swollen during fixation to form large granules, and the chondrioconts (filamentous forms) exhibited a series of swellings or became vesicular with chromophilic rims and chromophobic centers. Under such circumstances the chondriosomes resembled the dictyosomes of animal cells or the osmiophilic platelets as described by Bowen and Gatenby in plant cells. Guilliermond also observed the occasional anastomosis of the chondriosomes into a

network which was sometimes superimposed on the vacuolar system.

Zirkle (1932) studied vacuoles in primary meristem cells of *Osmunda*, *Lunularia*, *Pinus*, *Robinia*, *Phaseolus*, *Polygonum*, *Fraxinus*, and *Zea*, and noted that the reticulate, tannin-filled vacuole answered the requirements for a Golgi apparatus. That Zirkle did not believe the two structures homologous is indicated by the following quotation from his review of "The Plant Vacuole" (1937): "We have no right at present to speak of a specific Golgi material. Golgi himself described a "reticular apparatus" but later work has shown that the material in this apparatus need not always have a reticular form. We should remember, moreover, that the only reticular apparatus found thus far in the plant cell is the vacuole drawn out by the streaming protoplasm. It is obvious that before we may homologize the Golgi apparatus with any known structure in the plant cell, we will have to define the term much more precisely than we have thus far succeeded in doing."

METHODS OF DEMONSTRATION AND CHEMICAL COMPOSITION

Many of the controversies concerning the Golgi apparatus have arisen as a result of the quite different methods used by various investigators to demonstrate it.

Golgi bodies have been reported very infrequently in unstained living cells. Chadefaud (1937) described the peri-nuclear dictyosomes in unstained green algae; Gatenby (1930) stated that the Golgi region was visible in spermatocytes of crustaceans and molluscs; and plant vacuoles, of course, are often visible in living cells. Aside from these, there have been few observations of Golgi bodies in living cells; and osmiophilic platelets have not been so identified.

The methods most frequently used to demonstrate structures called Golgi bodies were: (1) impregnation with silver nitrate, (2) impregnation with osmic acid, and (3) vital staining. Early investigators usually limited themselves to one or, at most, two methods; but more recently all three have been used in some studies.

Methods involving impregnation with silver nitrate and osmic acid were very long and tedious. In general, both types involved preliminary fixation for from one to several days in certain cytological fixatives followed by immersion in solutions of silver nitrate or osmic acid for from one day to one month. By either of these procedures, the Golgi bodies were intensely blackened. The methods

of silver impregnation most frequently used, those of Cajal and Da Fano, were similar to the preparation of photographic negatives. Osmic impregnation methods in most common use were various modifications of the Mann-Kopsch and Kolatchev techniques. Raising the temperature to 35° or 40° C. during the period of impregnation was found to facilitate the demonstration of Golgi bodies. Bleaching with turpentine, potassium permanganate, or hydrogen peroxide was often resorted to in order to remove excesses of blackened precipitate and thus to differentiate the Golgi bodies from other cell constituents. The details of the methods of osmic and silver impregnation were compiled by Bowen (1928*a, b, c*), and can also be found in standard books on microtechnique.

Vital staining methods were used extensively by Dangeard (1923) to trace the development of the vacuome. Neutral red, used by Parat (1928) as a specific stain for the vacuome, was also applied to plants by Guilliermond (1929). He found that although the vacuome would stain with other vital dyes, such as Nile blue, cresyl blue, and methylene blue, neutral red entered the cells the most rapidly and was the least toxic of any. Guilliermond did not agree completely with Parat in believing that neutral red was a specific stain for the vacuome. However, he stated that aside from certain fat bodies mixed with free fatty acids, the vacuome was the only structure to absorb neutral red regularly. In that sense, neutral red might be considered as a specific stain for the vacuome.

Vital staining followed by one or both types of metallic impregnation was often used to establish the homology of structures with Golgi bodies. Hall (1931) exposed neutral red-stained flagellates to osmic acid vapor and fixed others by osmic and silver impregnation methods. Guilliermond (1929) noted that impregnation methods demonstrated patterns almost identical with those which could be obtained by vital staining with neutral red.

The general types of methods used by the principal investigators who studied Golgi bodies, and the results which they obtained are presented in the accompanying table. In this table the proponents of the osmiophilic platelet hypothesis are given first. Next below are those who incline toward the vacuome theory. They are followed by investigators who listed other types of structures as Golgi bodies, and by those who found no definite Golgi bodies in the cells studied. The table does not include all cytological methods used by

these investigators, but is limited to those which the particular persons considered Golgi methods.

Golgi methods have been criticized because of the length and complexity of procedures, frequently of failures, lack of specificity, and great variability of probable chemical background.

All fixation methods were described by Bowen (1928*b, c*) as capricious, subject to failure, and necessitating extreme care and constant personal experimentation and persistence. Failures were frequent; serious artifacts were not uncommon; and experience in cytology was essential to the proper judging of the success of an impregnation. Nevins (1933) found both silver and osmic techniques to be subject to failure. She stated that she had been unable to secure a perfect balance of the three factors which she believed to condition the results of osmic impregnation. These factors were: concentration of osmic acid, time interval, and carefully controlled temperature. Nahm (1933) observed Golgi bodies in only a small number of pieces of tissues fixed by the Kolatchev osmic method. Furthermore, she found Golgi bodies in relatively few cells of each successful preparation. Demonstration of Golgi bodies was found to be conditioned by four factors: the kind of tissue, the quality of the initial fixation, the position of the cell in the piece of impregnated material, and the temperature of incubation with osmic acid.

Guilliermond (1935) stated that Golgi methods were entirely unspecific and almost any cellular structure might be impregnated. As a rule, however, the silver nitrate was reduced most readily by the vacuolar content, and the osmic acid by the often much modified chondriosomes. The vacuome was the part most frequently stained by neutral red.

Golgi techniques have also been found to impregnate cellular components other than Golgi bodies. Savelli (1933) noted blackening of the leucoplasts of *Spiranthes* following immersion in silver nitrate. Weier (1938*a*) observed that "silver nitrate is reduced either as granules within the chloroplast, or uniformly along the edge of the chloroplast, or faintly throughout the stroma, or around the starch grains, or faintly throughout the cells of clover leaves depending upon the treatment of the leaves previous to impregnation with silver nitrate." Nevins (1933) noted that the Da Fano silver method produced so much indiscriminate blackening that it was impossible to distinguish any cellular constituents. Zirkle (1937) pointed out that historically the essentials of the usual Golgi

methods had first been used to stain the vacuoles of plant cells. He also observed that neutral red stained certain cytoplasmic granules as well as the vacuoles.

The chemical composition of Golgi material has been deduced from its reactions to fixing fluids and stains. Because of its ability to reduce osmic acid, Bowen (1928*b*) and Gatenby and Singh (1938) believed it to be lipoidal. Parat (1928) suggested that the vacuolar content might be a somewhat acidic solution sometimes containing protein. The reduction of silver nitrate was believed to indicate the presence of protein. Therefore, there was no agreement among investigators as to the probable chemical composition of the Golgi material, and theories concerning it have been shown to be based on inadequate evidence.

Hoerr (1936) began his report of some excellent experiments on the reaction of tissues to osmic acid with the following quotation from Heidenhain (1888): "Wie nicht alles Gold ist, was glänzt, so ist nicht alles Fett, was in Osmiumsäure dunkelt." Hoerr stated that Champy's fluid, used as the preliminary fixative in the Kolatchev method, is a very poor cytological fixative. He noted also that the osmiophilic and osmiophobic portions of the liposomes represented merely the results of incomplete penetration of osmic acid and were not due to differences in chemical composition of inner and outer portions. Pure lipin might be readily blackened with osmic acid and yet not blacken in the tissues. Another important point in connection with Golgi material was his statement that osmium reduced in tissues might migrate and be secondarily adsorbed by some other cellular structure.

Silver nitrate and osmic acid may be reduced by substances other than proteins and lipins. Zirkle (1937) stated that any vacuole which contained tannin could be blackened by both silver and osmium; any vacuole which contained chlorides would retain silver; and osmic acid might be reduced by lipoidal substances. Gautheret (1934) believed that vacuoles almost always contain colloidal materials such as proteins or tannin. He noted that cells of barley contained small intra-vacuolar bodies which stained with Sudan III and other fat stains, and postulated that these bodies were responsible for the blackening of the vacuome by Golgi techniques. Hoerr (1936) pointed out that blackening with osmic acid did not indicate the presence of lipin, but only of a reducing substance.

Neutral red, an indicator, has been used to demonstrate the

vacuome, or Golgi bodies of certain investigators. Neutral red is an indicator dye, red in acid solution and pale yellow in alkaline. Zirkle (1937) stated that neutral red did not accumulate in vacuoles with contents more basic than pH 6. Therefore, the ability to stain with neutral red indicates acidity, but gives no further information about chemical composition.

The Golgi apparatus was not easily or readily demonstrated by fixation, but usually required long and complicated procedures. These procedures demonstrated Golgi bodies in only a small number of cells. There were no specific stains for Golgi material, for all methods used also showed other structures but not always the Golgi bodies. The chemical composition of material designated as Golgi was not determined but was undoubtedly variable.

FUNCTIONS

The function most frequently assigned to the Golgi apparatus in animal cells was the formation of secretions. Although interpretations of the exact role it played varied considerably, many investigators agreed that the Golgi apparatus participated in the formation of whatever the cell synthesized.

One of the structures commonly believed to be the product of the Golgi apparatus was the acrosome (perforatorium) of animal sperm. Bowen (1928a) observed an "unmistakable relationship" between the osmiophilic platelets and the limosphere in androcytes of *Polytrichum*. The structural relationship seemed to him to indicate that the osmiophilic platelets secreted the limosphere, which was comparable with the acrosome of insect sperm. Parat (1928) assigned the function of secretion of the acrosome of the animal sperm to the vacuome. Weier (1931, 1932a) described a definite relationship between the Golgi-like plastid and the secretion of the limosphere in *Polytrichum* and *Catharinaea*, and postulated that the plastids performed a function which Bowen assigned to the osmiophilic platelets and Parat to the vacuome. On the other hand, Nevins (1933) found no evidence to warrant the conclusion that any cytoplasmic constituent (mitochondria, plastids, vacuoles, or osmiophilic platelets) played a direct role in the formation of the limosphere of *Sphaerocarpos*.

Aside from the formation of the limosphere little evidence of secretory activity of the Golgi apparatus in plant cells has been presented. This may be due, at least in part, to the fact that the majority of the investigators of Golgi bodies have used meristematic

tissues. Bowen (1928a) complained that the osmiophilic platelets of meristem cells exhibited an apparent "lack of interest" in cellular function. Nevins (1933) did not observe a relationship between the Golgi bodies, or the osmiophilic platelets, and the formation of secretion in nectar glands of flowers. Weier (1932b) compared the elaboration of starch by the chloroplast (Golgi apparatus) with the formation of secretory products in animal cells. He suggested that the function of the "plastid cytoplasm" was the secretion of enzymes involved in photosynthesis. Sass (1934) observed that the Golgi crescents formed by the division of the Nebenkern (Golgi apparatus) moved into the apex of the basidium where they became associated with the developing sterigmata and spores.

Scott (1929) suggested that the osmic acid-reducing substances were food reserves. She observed that these materials were present in meristematic cells of very young roots, disappeared as the first secondary roots appeared, and then reappeared when the secondary roots were one-half inch long. She interpreted this as indicative of a utilization of the reserves (osmiophilic material) during the formation of branch roots.

Gatenby and Singh (1938) assigned an osmo-regulatory function to the Golgi apparatus of *Copromonas* and *Euglena*. To support their theory they described a regular series of changes in the Golgi bodies applied to the contractile vacuole as it changed from systole to diastole, and *vice versa*. Gatenby, Singh, and Browne (1938) postulated that the Golgi apparatus of the Protozoa persisted in higher animals, and as the osmo-regulatory mechanism (contractile vacuoles) characteristic of the Protozoa disappeared, the Golgi bodies became concerned with the removal of water from secretion products formed in close proximity with them.

It is of interest to note that aside from Weier, the majority of those who assigned specific functions to the Golgi apparatus were zoologists who had turned their attention to plant cells and sought functions comparable with those believed to occur in animals.

THE PRESENT STATUS OF THE GOLGI QUESTION

During the last five years the discussions of Golgi bodies, so vigorous between 1926 and 1933, have subsided somewhat. Most recent papers on structure of plant cells do not list Golgi bodies as cellular entities or as homologues of any other cellular structure.

Dangeard (1935) grouped cellular constituents as follows: (1) "nucleome," composed of all nuclear constituents; (2) "plasti-

dome," made up of all the plastids; (3) "vacuome," composed of the vacuoles; (4) "chondriome," the chondriosomes; and (5) "ergastome," composed of lipoidal droplets and other structures which arise *de novo*. Golgi bodies were not mentioned in this paper.

Guilliermond (1935) withdrew the vacuome theory and concluded that Golgi bodies did not exist as separate entities in plant cells. He based his conclusion on a large number of personal observations of plant cells and included, in connection with his descriptions and discussion, a review of the literature concerning the Golgi bodies. Guilliermond interpreted structures which had been called Golgi bodies by other investigators as follows: Drew's Golgi bodies were chondriosomes; the osmiophilic platelets of Bowen and Gatenby were plastids and vesicular chondriosomes which had been altered by the fixation methods used to demonstrate them; Weier's hypothesis was entirely unacceptable because plastids were found exclusively in green plants and were, therefore, not of universal occurrence as Golgi bodies, of necessity, must be; in Protozoa the Golgi bodies were either vacuoles or chondriosomes. Guilliermond also criticized zoologists for their lack of vital observations and for their tendency to identify morphologically different structures, *e.g.*, nets and dictyosomes, as Golgi bodies when the only criterion for their identification was the use of admittedly non-specific impregnation methods.

Guilliermond (1937) described an additional series of observations on materials prepared by what he designated as convergent techniques. The procedures included were studies of: (1) fixed preparations; (2) living unstained cells; (3) vitally stained materials; (4) cytochemical analyses; (5) cytophysical analyses including viscosity, osmotic pressure, *etc.*; and (6) cellular structures at all stages of their life cycles. He deplored cytologists' regrettable habit of arriving at general conclusions from particular facts observed in more or less favorable special types of cells. He emphasized the fact that the problem of modern cytology was to find the relationship between cellular structures and their physiological activities. Guilliermond believed that his convergent methods would be valuable because they made possible the distinction of such structures as chondriosomes, young plastids, and young vacuoles, which at some stages were quite similar. Guilliermond did not mention Golgi bodies in this article but did state that the vacuoles were stained with neutral red.

Buvat (1937) identified lipoidal inclusions in the vacuoles of meristem cells of castor bean, broad bean, pea, and a cucurbit, and regarded them as readily available reserves. In cells fixed by mitochondrial methods and then treated with "Sudan B noir," the lipid bodies had undergone changes analogous to those obtained by osmic impregnation and had come to resemble Golgi bodies. The inference is that Buvat did not consider Golgi bodies distinct cellular entities.

Duchaussoy (1937) followed the history of the chondriosomes and vacuoles from the germination of the spore to the origin of basidium and basidiospore of *Coprinus*. He did not identify any structure as the Golgi apparatus, although his figures showed vesicular chondriosomes which resembled osmiophilic platelets in form.

Sorokin (1938) observed living epidermal cells of *Allium cepa* but listed no cytoplasmic structures other than mitochondria and plastids.

Zirkle (1937) expressed the belief that many of the inferences of those who had described the Golgi apparatus were "reared on an insufficient factual basis," and concluded that the identification of a Golgi apparatus in plant cells is largely a matter of definition.

Weier (1938*a, b*) made no statements concerning his earlier idea of a plastid-Golgi homology, but supported the hypothesis, reviewed by Weber (1937), that ascorbic acid was responsible for the reduction of silver nitrate by plastids.

The osmiophilic platelet hypothesis was upheld by Beams and King and Jones on the basis of evidence obtained from a study of cells fixed by Golgi methods after centrifuging. Beams and King (1935) summarized the evidence which seemed to support this theory as follows:

"1) The osmiophilic platelets, in many cells at least, are impregnated by osmic acid methods (Kolatchev and Mann-Kopsch Weigl) commonly used to demonstrate the animal Golgi apparatus.

2) The osmiophilic platelets resemble both form and structure (osmiophilic cortex and osmiophobic medulla) of certain insect Golgi bodies.

3) Bowen claims to have shown that the function of the osmiophilic platelets in spermatogenesis of certain plants is similar to that of the Golgi bodies in animal spermatogenesis.

4) Neither the Golgi apparatus nor the osmiophilic platelet stains with neutral red.

5) As has been shown in this paper, the osmiophilic platelets in the root tip of the bean move to the centripetal pole of the cell, upon centrifuging, as does the Golgi apparatus of animal cells." It may be noted in passing that platelets are not "osmiophilic" unless they reduce osmic acid. Jones (1938), working in Gatenby's laboratory, mentioned only one discrepancy between her work and that of Beams and King, and that was in the position of the osmiophilic platelets and the pseudochondriome with respect to the cytoplasm. Jones reported the pseudochondriome in the area between the osmiophilic platelets and the cytoplasm. Pseudochondriome and osmiophilic platelets, therefore, formed adjacent strata in the cell; but Beams and King observed the pseudochondriome centrifugal to the cytoplasm and separated by it from the osmiophilic platelets. Furthermore, Jones did not succeed in demonstrating pseudochondriome and osmiophilic platelets simultaneously. In composite figures based on both Kolatchev and Champy-Kull preparations, she showed osmiophilic platelets and pseudochondriome adjacent to each other and almost identical in size and form. Chondriosomes as well as osmiophilic platelets frequently blacken with osmic acid [see Bowen (1928a) and Nahm (1933)], and even if the cell were extraordinarily uniform in size and shape, it seems unlikely that such a fine distinction should be made.

Beams and King (1939) concluded that the osmiophilic platelets constituted a distinct category of cellular structures, but they did not seem quite certain with which other categories they should be related.

Northen (1936) called all small inclusions "mitochondria" and did not attempt to distinguish between chondriosomes and osmiophilic platelets. He observed that the "mitochondria" were displaced with the cytoplasm when cells were centrifuged and concluded that the "mitochondria" and cytoplasm were of like specific gravity. Beams and King (1939) stated that the centrifugal force used by Northen was insufficient to distinguish between the specific gravities of the cytoplasm and the "mitochondria" (pseudochondriome and osmiophilic platelets of Beams and King).

The Golgi material of flagellates, according to Hall (1936), consisted of numerous small scattered globules which could be stained vitally with neutral red and became impregnated with osmic acid or silver nitrate. He believed that any substance which was considered to be Golgi material must be demonstrable by all three methods and

be similar in general form in all flagellates. On the other hand, Gatenby, Singh, and Browne (1938) identified a Golgi apparatus surrounding the contractile vacuole of flagellates solely on the basis of its ability to blacken with osmic acid. The fact that it did not stain with neutral red and was comparable in general morphology with the Golgi bodies of certain metazoan cells constituted, for them, proof that it was the true Golgi apparatus. It is apparent, therefore, that the question of Golgi bodies in the flagellates is still unsettled.

The present status of our information concerning the Golgi bodies of plant cells may be summarized as follows: Guilliermond has withdrawn the Golgi-vacuome homology hypothesis; Beams and King and Jones support the osmiophilic platelet hypothesis; both scattered globules and osmiophilic surface of contractile vacuoles have been identified as Golgi bodies of flagellates; and the majority of recent investigators of plant cells have not listed Golgi bodies as distinct cellular entities.

SUMMARY

1. The methods commonly used to demonstrate Golgi bodies have been metallic impregnation with osmic acid or silver nitrate and vital staining, usually with neutral red.

2. Using these methods investigators have identified the following principal types of Golgi material in plant cells: osmiophilic platelets, the vacuome, small scattered globules, the cortex of the contractile vacuoles, and plastids.

3. Depending upon the methods used to demonstrate it, the Golgi material has been assumed to be a lipid, a protein, or an acidic aqueous solution. Other evidence indicated that probably none of these was entirely correct.

4. Functions which were assigned by different investigators to structures which they identified as Golgi bodies in plants included: *a*) secretion of the limosphere of moss androcytes; *b*) secretion of enzymes involved in photosynthesis; *c*) contributions to the formation of sterigmata and basidiospores; *d*) osmo-regulation in flagellates; and *e*) storage of food.

5. The vacuome-Golgi hypothesis was withdrawn by Guilliermond in 1935. The osmiophilic platelet hypothesis is still upheld by a few investigators. The majority of recent studies of plant cytology have not included Golgi bodies as morphological entities.

COMPARISON OF METHODS AND RESULTS

Tissues were from spermatophytes, unless otherwise specified. Under METHODS USED, "osmic" indicates well known osmic impregnation methods; "silver" indicates silver impregnation methods; "neutral red" indicates vital staining with neutral red; "vital staining" indicates staining with more than one vital dye; techniques not commonly used for Golgi bodies are indicated by name. PARTS IMPREGNATED OR STAINED include those observed to be regularly or occasionally impregnated with silver or osmic acid or stained by vital dyes. Under STRUCTURES IDENTIFIED AS GOLGI BODIES, a question mark indicates some doubt as to the exact meaning of the author.

INVESTIGATOR	PLANT OR TISSUE STUDIED	GOLGI METHODS USED	PARTS IMPREGNATED OR STAINED	STRUCTURES IDENTIFIED AS GOLGI BODIES
Drew	meristem	silver chrome-osmic	not successful oval and elongated bodies	blackened batonettes
Bowen	male heads of <i>Polytrichum</i> ; meristems of <i>Equisetum</i> and spermatophytes	osmic silver	osmiophilic platelets, pseudochondriome, plastids, vacuoles not successful	osmiophilic platelets
Patten, Scott, and Gatenby	meristem	osmic silver	osmiophilic platelets, mitochondria not successful	osmiophilic platelets
Beams and King	meristem	osmic after centrifuging	osmiophilic platelets, protoplasts, pseudochondriome	osmiophilic platelets
Jones	<i>Elodea</i> leaves; meristem	osmic silver	osmiophilic platelets, plastids heavy precipitate	osmiophilic platelets
Gatenby, Singh, and Browne	flagellates	osmic silver	osmiophile layer around contractile vacuole or reservoir not successful	osmiophile cortex of contractile vacuole or reservoir

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INVESTIGATOR	PLANT OR TISSUE STUDIED	GOLGI METHODS USED	PARTS IMPREGNATED OR STAINED	STRUCTURES IDENTIFIED AS GOLGI BODIES
Dangeard, P. A. and Pierre	meristems; fungi; yeast; etc.	vital staining vacuome	vacuome	not specifically stated
Bensley	meristem	none	vacuoles demonstrated	vacuoles
Guilliermond	meristem	silver neutral red	vacuome vacuome	vacuome (later retracted)
Parat	animal tissues	neutral red	vacuome	vacuome
Scott	meristem	Bensley's osmic	vacuome and diffuse blackening	vacuome?
Bose	agarics and polypores	osmic neutral red	blackened nets from vacuoles vacuome	vacuome vacuome
Gonçalves da Cunha	stages in maturation of wheat grains	silver	vacuome and chromatin	vacuome
Hall	flagellates	neutral red osmic and silver	vacuome vacuome and many other cell parts	vacuome
Cassaigne	<i>Saprolegnia</i> , yeast	neutral red	vacuoles	?
Py	tapetal cells	osmic; silver	vacuome and mitochondria	vacuome?

COMPARISON OF METHODS AND RESULTS

Tissues were from spermatophytes unless otherwise specified. Under METHODS Used, "osmic" indicates well known osmic impregnation methods; "silver" indicates silver impregnation methods; "neutral red" indicates vital staining with neutral red; "vital staining" indicates staining with more than one vital dye; techniques not commonly used for Golgi bodies are indicated by name. PARTS IMPREGNATED OR STAINED include those observed to be regularly or occasionally impregnated with silver or osmic acid or stained by vital dyes. Under STRUCTURES IDENTIFIED AS GOLGI BODIES, a question mark indicates some doubt as to the exact meaning of the author.

INVESTIGATOR	PLANT OR TISSUE STUDIED	GOLGI METHODS USED	PARTS IMPREGNATED OR STAINED	STRUCTURES IDENTIFIED AS GOLGI BODIES
Weier	cells during sporogenesis and spermatogenesis of mosses	osmic silver	rim of plastid not successful	plastids
Hovasse	Volvocales	silver; osmic	vacuole, chondriosomes, dictyosomes	dictyosomes
Chadefaud	green algae	neutral red Zenker-formol living unstained	physodes chondriosomes, dictyosomes	dictyosomes
Dubosq and Grasse	flagellates	neutral red; osmic	food vacuoles, contractile vacuoles, granules, vacuoles, parabasal granules	parabasal apparatus
Sass	pileus of <i>Coprinus</i>	none	Nebenkern, Golgi crescents, minute granules	Nebenkern and Golgi crescents
Kiyohara	meristem; leaves; potato tuber	osmic; silver	plastid margins	none
Nevins	antheridia of <i>Sphaerocarpos</i> ; nectar glands	neutral red	not successful	none
		silver osmic	black granules or globules osmiophilic platelets, vacuoles	
Zirkle	meristems of ferns and spermatophytes	silver; osmic	vacuoles	none

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THE STRUCTURE OF THE CONE IN THE CONIFERAE

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There is at present much difference of opinion on the structure and organisation of male and female cones in the Coniferae. The writer has come to the conclusion that the differences have arisen because the various bases upon which a solution has been sought have not been broad enough, and that one is available if due consideration is given to certain deductions from Hofmeister's (1849-51) classic comparative researches. In these, Hofmeister proved the relationship of the seed habit of higher plants to the free-sporing habit of vascular cryptogams by his interpretation of the embryo sac of the Coniferae "als eine Spore welche von ihrem Sporangium umschlossen bleibt" (1851, 151), thus demonstrating that both male and female cones were ancestrally free-sporing, and premising homology between them at least in the fundamental features of their organisation. There is certainly no evidence in either homosporous or heterosporous vascular cryptogams, fossil or living, that would lead one to suspect any fundamental difference in their male and female sporophylls; and, as a matter of fact, homology between male and female sporophylls has long been recognised in the most primitive of living seed plants, the Cycadaceae.

Granting such homology in the ancestral conifers, this feature should be given basic consideration in the interpretation of male and female cones of their descendants, the living conifers of today. This is not a new idea but, though subsequently overlooked, was suggested over a hundred years ago by the distinguished exponent of gymnospermy, Robert Brown. Acceptance of this idea makes two other points clear: first, that the evidence of homology should be most apparent in the most primitive conifers; second, that the male cone having retained the free-sporing habit and being therefore more primitive than the female should show the fundamental structural features of cone organisation more simply and more clearly than the female.

In the light of these deductions from Hofmeister's work, the

necessity of a thorough knowledge of the male cone as a basis for interpretation of the female becomes evident, as also the necessity of an acceptable explanation of the greater specialisation of the female cone. On the latter point the evolution of sexual differentiation in the gametophyte sheds significant light. Here the course of evolutionary sequence indicates that there has been progressive sexual differentiation beginning with the gametes and their differentiation, and extending outward from them to the gametangia and prothallia, as indicated by the successive acquirement of heterogametangy and heterothally, whether the spores are exosporal or endosporal in their prothallial development. On acquiring the seed habit the female spore was retained within its sporangium and derived all its nutrition from the surrounding tissue for the whole of its endosporal prothallial development from the uninucleate stage onward, including fertilisation and embryo development. We have thus in the female cone an accentuated influence spreading from the female spore and prothallium to the sporangium and then to the structure on which it is borne, the sporophyll. With regard to the second point, the relative primitiveness of the male cone compared with the female, there are many difficulties. These are associated with the fact that three different types of stamen, the *Taxus*, *Pinus* and *Araucaria* types, are regarded as primitive by different botanists.

Under the circumstances it was necessary to investigate the cone and stamen of the whole conifer series anew, and since the Taxineae presented the most difficult problems these were studied in most detail. The results may be summarised as follows: (1) that the stamen of *Taxus* is not primitively peltate and radially symmetrical but that this appearance is due to the fusion of dorsiventral sporophylls, usually of two but sometimes of three; (2) that similar fusion occurs at the apex of the cone in every family of the conifers but rarely at other parts except in the Taxineae; and (3) that in *Austrotaxus* there is an additional fusion, that of the cone to the subtending bract.

In general it may be said that wherever fusion of sporophylls occurs it is either lateral or back-to-back depending on whether the sporophylls are spirally arranged or in opposite series. Since fusion usually occurs at the apex of small cones where the axis contracts and there is aggregation of the sporophylls, it is consid-

ered that these are predisposing factors. In *Taxus* the aggregation is extreme, the stamens being massed in a small head at the apex of the cone axis, their pollen sacs fused into a synangium. Another feature of the apical sporophylls in many forms is elimination of the lamina. This condition is characteristic of *Taxus* and results in projection of the pollen sacs beyond the stalk to which they are laterally attached. In other small cones the projection is often quite marked, particularly where bud scales replace the lamina in protection of the cone. In many cases complete or partial reduction of the lamina (the latter being characteristic of the apical region of all conifers) is accompanied by decrease in the number of pollen sacs borne by these terminal sporophylls when compared with the number on the lower sporophylls of the same cone, e.g., from four to one in *Tetraclinis* and from three to one in *Juniperus* and *Thuja*. In no case is this reduction of sporangia accompanied by increase in the number of resin cysts, as required by the 'sterilisation' theory of Coulter and Land (1905), but more often by their reduction or elimination. In fact, reduction of the one is generally correlated with reduction of the other, this being particularly evident when the variation is great, as in the Araucarineae. Thus this theory upon which the suggested derivation of the dorsiventral stamen from the *Taxus* type depends, lacks confirmation of its basal postulate—*increase of resin cysts where there is reduction of sporangia*.

Austrotaxus affords further evidence that fusion plays an important rôle in the organisation of the male cone. Here the cone is fused throughout its entire length with the subtending bract, and bears normally five sporophylls on its free surface, two of which are located laterally at the base of the cone, have a much reduced lamina and bear two sporangia each, while the three distal sporophylls have no lamina and bear one projecting sporangium each. The only fused sporophylls are the two most distal. The related genus *Cephalotaxus* shows partial fusion of the cone to the bract. That this represents a more primitive condition is indicated by other features of its cone organisation (more sporophylls to a cone, less reduction of the lamina, more sporangia to a sporophyll, less fusion of terminal sporophylls). Though it is recognised that fusion of floral parts in angiosperms is indicative of specialisation it is conceivable that this might not be so in conifers. Interpretation in the

reverse way, however, would involve the origin of the dorsiventral stamen from the *Taxus* type. That it has not originated by 'sterilisation' of sporangia has already been shown, and its derivation by splitting would imply an interpretation of the concentric vascular bundle of the *Taxus* stamen, which would be difficult to accept since the occurrence of this most primitive type of bundle if not the result of fusion of dorsiventral bundles would imply that the stamen of *Taxus* is more primitive than that of the cycads or *Ginkgo* where the vascular bundles of the sporophylls are normally collateral and dorsiventral.

The preceding considerations have led to the conclusion that the dorsiventral type of male sporophyll, which characterises the great bulk of the conifers, is the primitive and basic conifer type, reduction and fusion indicating the lines along which specialisation has taken place. Which of the two dorsiventral types, that of *Pinus* or that of *Araucaria*, is the more primitive will be more advantageously discussed after consideration of current theories of cone structure in connection with which certain evidence arises which has a bearing on the question. These theories are of three types: one, based on homology between radially symmetrical male and female sporophylls; another, the ligular, in which homology of dorsiventral sporophylls is involved; and a third, the brachyblast, which regards the male and female cones as differing in organisation.

The theories based on radial symmetry have been strongly supported by various botanists (Čelakovský, 1897; Doyle and O'Leary, 1934; and Hirmer, 1936). One of the main objects of these theories has been to provide an acceptable explanation of the location of male and female sporangia on opposite sides of homologous sporophylls, a point, however, which is capable of an equally simple explanation when the location of the male and female sporangia in the cycads is taken into account. In support of their basic requirement, radial symmetry, these theories cite the *Taxus* type of stamen as the most primitive in living conifers. In view of the conclusion arrived at in the present study that the *Taxus* type of stamen results from fusion of primitive dorsiventral sporophylls, it is clear that these theories must depend on other evidence for their substantiation. Such other evidence as has been presented, however, is unconvincing, dealing as it does with external morphological features

of abnormalities in living conifers and with the questionable presence of radial symmetry in the postulated ancestral forms.

The ligular theory, on the other hand, though it implies homology between the sporophylls of male and female cones, does not attempt to explain the reversed location of male and female sporangia. It also ignores certain other implications of homology. For example, it fails to account for the absence of a ligule in the male cone where homology would suggest its presence. Here, if the primitiveness of the free-sporing habit is taken into account, the ligule should be more evident than in the female. Again, on the basis of the ligular theory the conifers are usually regarded as derived from such ligulate lycopsid forms as *Selaginella* where there are ligules, not only on the micro- and mega-sporophylls but on the vegetative leaves as well, making comparison with the conifers still more difficult. In the case of these ligulate Lycopsida there is another feature, difference in spore size, which distinguishes them from the conifers. All ligulate Lycopsida produce spores of two sizes, very small and very large, whereas in the conifers and in fact in all seed plants the spores are not significantly different in size (Thomson, 1927). Homospory and not heterospory is thus basic in the ancestry of the conifers. That such homosporous ancestry should be sought in the Pteropsida instead of in the Lycopsida is apparent from the work of Jeffrey in which he demonstrated that the conifers belong to the Pteropsida because they invariably have leaf gaps, no matter how small the leaves, while in the Lycopsida no such gaps are present. It is on these facts that the comparison which Seward (1919, 117) made between the organisation of the female cone in the Coniferae and that of *Lycopodium* must not be considered as indicative of their ancestry. His comparison with *Lycopodium*, however, is important in that it indicates that the ligule, not being present in *Lycopodium*, cannot be homologous with the outgrowth associated with the ovule in the Coniferae.

The brachyblast theory, unlike the ligular and also unlike the one here proposed, does not admit that the male and female cones are homologous but regards the female cone as one grade higher in organisation than the male—an 'inflorescence' instead of a 'flower.' This interpretation of the female cone is based on the presence of an upper series of inversely oriented bundles which is considered to indicate that the ovule-bearing structure is a short-shoot or

brachyblast arising in the axil of a bract. Thus on the basis of the brachyblast theory those conifers which show the greatest development and independence of the ovuliferous scale are regarded as most primitive. These are included in the Abietineae which in anatomical structure have the greatest array of specialised features of any family of the Coniferae—highly developed systems of ligneous resin canals, both vertical and horizontal, the latter extending into the bast with bulbous expansions capable of continued growth; ligneous rays with very abundant and sometimes elaborately sculptured ray tracheids; tracheids with pitting provided with specialised “rims of Sanio,” *etc.* This statement is not intended to imply that there is of necessity a phylogenetic correlation between anatomical structure and cone organisation, but only that any theory which is corroborated by such evidence should be given preference over an equally acceptable one which lacks such corroboration. On this basis the ligular theory takes precedence over the brachyblast, since it accords primitive place in cone organisation to the Araucarineae, forms which at the same time have very simple anatomical structure of their wood—none of the specialised features characteristic of the Abietineae. Viewed from this standpoint the ligular theory provides evidence that the dorsiventral araucarian type of stamen (multi- and free-sporangiate) is more primitive than that of the pines (bisporangiate, and the sporangia fused with the filament).

Strong support of this view was presented by Thibout (1896) in his comprehensive study of the male cone of gymnosperms, from which he concluded that the ‘cycad-araucarian’ type of stamen was the most primitive in the Coniferae, a study which has not received the attention it deserves. Additional evidence that the araucarian type is primitive is provided by one of the deductions from Hofmeister’s work, that homology between the male and female cones is most clearly expressed in the most primitive forms. Since the extension of sex-differentiated features proceeds outwardly it follows that resemblance in external features becomes a valuable criterion of primitiveness. Seward was the first to apply this principle to the conifers where similarity in both size and external morphology of the male and female cones and sporophylls of the araucarians is in some cases almost as marked as in the cycads, a condition which is not approached in any other conifer family.

In both cycads and araucarians there is also similarity in the

structure of the male and female cones, in that both show a functional correspondence between the upper and lower inversed vascularisation of their respective dorsiventral male and female sporophylls which is associated with the vascular supply of their sporangia. In the araucarians the sporangial-supply nature of the lower in the male is evident from the fact that the number of bundles varies directly with the number of pollen sacs (from 23 to 2 as observed). On the other hand, in the female cone with its uni-ovulate sporophylls the variations are correlated with size of the seed, the differences in which are very marked. Where the seed is small, as in *Agathis*, its inversed supply is small and detaches itself from that of the sporophyll near the base of the seed; where the seeds are progressively larger in different species of *Araucaria* their supplies are correspondingly larger and detach themselves progressively nearer the axis of the cone; and in *A. bidwilli* where the seed is largest the supply is largest and there is direct attachment to the vascular bundles of the cone axis. These facts coupled with the finding of four ovules on a scale of *Zamia*, two of which were on the upper surface (as in the conifers) and supplied entirely by inversed bundles, finally made it evident that the inversed upper supply is not indicative of a brachyblast, but that both upper and lower are of sporangial-supply nature and so more comparable to the inversions characteristic of 'enations.'

Viewed from the standpoint of sporangial supply, it is evident that if the conifers are monophyletic, as indicated by the organisation of the male cone, the female should show basic similarity of organisation throughout the whole group. In this connection importance attaches to the number and size of the ovules and also of their associated protective structures, the ovuliferous scales (a designation appropriately applied by Seward to the so-called ligule of *Araucaria*). When so interpreted it becomes evident that the living conifers represent two divergent lines of development, one with a single ovule to the sporophyll and the other with several. If these two lines are associated with the araucarians in ancestry both ovular conditions must be present in their ancestral forms. Far back in the fossil record there are sporophylls ascribed to the araucarian conifers bearing one, three and five ovules, each ovule with an ovuliferous scale more or less developed. That the uni-ovulate condition of living araucarians is closely associated in an-

cestry with the tri-ovulate is indicated by the work of Mitra (1927) on *Araucaria* in which there is both morphological and anatomical evidence that two extra (lateral) ovules and ovuliferous scales had aborted, while in *Agathis* vestigial lateral inversed bundles suggest abortion of two ovules in this genus (Thomson, 1906). That there is a similar relationship between the five- and the three-ovulate conditions may be inferred from Walton's (1928) description of *Voltzia Liebeana*. Here there are three large and two small scales attached proximally to the sporophyll at their united bases. The large ones are each considered to have had an associated seed as indicated by detachment scars, and since in the living forms one ovuliferous scale to the ovule is the rule and this is smaller if the ovule aborts it is probable that this fossil form bore two extra ovules which aborted early. Thus in the living and fossil araucarians there is evidence of the basis necessary for the related origin of the two lines of development as seen in the living conifers, lines which may be appropriately designated taxacean and pinacean since they culminate respectively in *Taxus* and *Pinus*.

In the taxacean line, where the uni-ovulate condition is basic, specialisation shows itself in reduction of the number of sporophylls to a cone and the gradual loss of community protection, such as that provided in the araucarian sporophyll type of cone. An early stage is illustrated by *Saxegothaea* and *Microcachrys*, in the latter of which one ovule may early take precedence over the others and be the only one to mature (Thomson, 1909). Accompanying reduction in the number of sporophylls to a cone there is gradual attainment of individual protection of the single ovule as it increases in size by acquirement of a thick, sclerotic (sometimes fleshy) testa, and by differentiation of the ovuliferous scale into a fleshy arillus, as seen in the higher Podocarpaceae and Taxineae. The stages of differentiation of the ovuliferous scale are gradual, beginning with its broadening and partial encirclement of the ovule in the lower Podocarpaceae where it is unvascularised, and ending with complete encirclement and vascularisation in the higher forms, where the structure of the arillus in *Taxus* indicates that several infertile ovuliferous scales are concerned in its formation, a condition similar to that in the ovule as described by Miss Aase (1915). In the taxacean line the inversed ovular supply, which in the lower forms

is derived from the normal sporophyll bundle, soon becomes axial in origin as the ovule becomes larger and more axially located.

In the pinacean line, the sporophylls bear several ovules each and there is community protection of the ovules, at first (as in the araucarians) almost entirely by the sporophylls themselves. This sporophyll type of cone is found in the most primitive Taxodineae. Here the ovuliferous scales (one to each ovule) are small and unvascularised, their number varying with the number of ovules, each of which is supplied by one inversed bundle (3 in *Cunninghamia* and 3 to 6 in *Athrotaxis selaginoides*). Further advance involves shifting of part of the protective function from the sporophylls to the ovuliferous scales which become larger and vascularised, each from the bundle supplying its ovule. These ovuliferous scales are independent of one another and of their sporophyll in the young stage but later fuse at their bases with each other and with their sporophyll, growth in this region producing a combination type of scale with its component parts (fused ovuliferous scales and sporophyll) showing as free tips when the cone is mature. This combination type of structure is illustrated in *Cryptomeria* where the number of ovules and ovuliferous scales per sporophyll was found to vary from 3 to 6 with corresponding variation in their vascular supplies. *Sciadopitys* shows a more extreme variation in the number of ovules, ovuliferous scales and bundles (5 to 13). In the Cupressineae, where the ovules also vary greatly in number but are attached closer to the cone axis than in the Taxodineae, fusion takes place so early that at maturity scarcely a trace of the individuality of the different parts is evident. That the ovuliferous scale in the higher Taxodineae and Cupressineae is of the fusion type, is indicated in the lower forms, where the number of ovules is large, by correspondence between the number of inversed bundles and ovules at an early stage, the lack of such correspondence at a later stage in the lower and in all stages of the higher forms being probably associated with early abortion of ovules, chiefly of the lateral. Whatever the origin of the increase in the upper series it is correlated with decrease in the normally oriented lower, from the large number in the Araucarineae to one in most of the higher Taxodineae and Cupressineae, a change which is associated with the gradual transfer of the protective function from the sporophyll proper to its fused ovuliferous scale component, and with the more

axial origin of its vascular supply. Finally, in the Abietineae, where the two components of the sporophyll are almost separate, the transfer of the protective function to the upper becomes practically complete, the lower sharing in this usually only when the cone is very young, and acquiring a new function—that of aiding (by uprolling of its margins) to separate the ovuliferous scales for the access of pollen. In the resultant fused-ovuliferous-scale type of cone, the individual scale has much more vascularisation than required for the supply of its two ovules, a supply which in the upper part of the cone may make as many as five separate attachments to axial bundles. That the central part of the extra vascularisation of the scale is associated with the abortion of an ovule is suggested by Miss Aase's (1915, 289) work on *Keteleeria* in which she found evidence of a third (abortive) ovule between the other two, a feature that may also have a bearing on the origin of the keel which, like the sporophyll in the young stage, aids the access of pollen. In the Abietineae, however, there is no similar evidence on the origin of the extra lateral bundles, but the conditions referred to in the Taxodineae and Cupressineae are suggestive. In the pinacean line, as in the taxacean, the inversed ovular supply bundles in the lower forms arise by division of the normally oriented bundles of the sporophyll, and in the higher independently from bundles of the cone axis, but whereas in the uni-ovulate line the transition is abrupt accompanying the more rapid change in size of the seed and reduction of the cone, in the pluri-ovulate line where the number instead of the size of the ovules is important and the reduction of the cone of significance only in the higher Cupressineae, there are more intermediate forms.

Such intermediate forms not only show the general trend of specialisation very clearly but the individual cone of certain of them may also show a similar trend, repeating the group sequence more or less completely from base to apex of the cone. It was, for example, at the base of the cone that Miss Aase found indications of tri-ovulate scales in *Keteleeria*, and Mitra a similar condition in *Araucaria*. There is also clear evidence of this trend in the source of the upper vascularisation which in basal scales may arise in the primitive way by division of the normally oriented sporophyll bundles and towards the apex from those of the axis as in the specialised forms. In assessing the significance of this change of

origin, infertility might seem to be involved, but this cannot be the determining factor since the origin is still axial in infertile scales toward the apex of the cone. In the male cone where the requisite intermediate degree of specialisation is more often found than in the female, the trend is of more general occurrence and more clearly expressed than in the female cone. This trend is exceptionally clear in the common type of bi-sexual cone in which the more primitive (male) sporophylls are borne at the base and the more specialised (female) above, an explanation which is equally applicable to the angiosperm flower. In the basal region of the female part of such cones the sporophylls may bear ovules on the lower surface, a reversion to their original position as indicated by the normal location of the pollen sacs of the more primitive male sporophyll. In other cases the ovules in this region may be laterally located, as in cycads, and so show partial attainment of their normal location in the upper part of the cone. A similar primitive location of ovules may occur in the case of sporophylls from the base of wholly female cones.

On the basis established by Hofmeister's work it is evident that in the conifer series specialisation of the male and female cone structure has followed two distinct lines, the specialisation in these lines arising from sex-associated differentiation of homologous dorsiventral sporophylls of araucarian type and culminating in strikingly different ways. In the final stage of the taxacean line both male and female cones are much reduced and have fused sporophylls, the female with a single apical ovule and elaborate individual protective structure, while in the final stage of the pinacean line both sporophylls have attained a stabilised two-sporangiate condition with the sporangia in the male grown to the filament and in the female partly embedded in the highly developed ovuliferous scale, the aggregate of such scales forming the most specialised community type of ovular protection in the conifers. In the course of specialisation of the male and female cones in both lines reduction and fusion play such significant rôles that they assume an importance somewhat commensurate with that accorded them in connection with the flower of the angiosperms.

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MITOCHONDRIA IN PLANTS¹

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INTRODUCTION

The study of mitochondria in recent years has come to occupy an interesting position in biology. Despite their comparatively recent discovery and tentative description as cell organs *sui generis*, accepted now by many cytologists, an extensive literature has accumulated on the subject, as perplexing and conflicting as it is abundant. This lack of agreement among the witnesses is not so difficult to understand, however, since mitochondria, though ubiquitous, are often insignificant in appearance and truly protean in morphology and behavior.

It has been suggested that the comparatively recent discovery of mitochondria and the possibilities thus presented by a new category of cell organs for the explanation of hitherto obscure cellular functions have animated many workers and led them to conduct hastily arranged and brief investigations on the functions of these structures. And Harper (134) characterized the work of the early investigators of mitochondria as a mere tabulation of the appearance of variously fixed and colored particles in the cell body with the hope that such bodies might later be found to be specific and fundamentally significant.

Further explanation for the lack of consonance among the investigators may be had in the confusion in terminology resulting from the frequent introduction of a new name inspired apparently by morphological appearance or supposed physiological function of bodies often not mitochondrial in nature. The resulting redundancy has made accurate appraisal difficult.

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Their late appearance in cytological literature, as well as much of the subsequent misunderstanding concerning them, is largely attributable to the use of unfavorable killing fluids so long in vogue. The early and sustained dominance of the nucleus and nuclear phenomena in the interests of cytologists resulted in the use of killing fluids which partially or totally destroyed the cytoplasm and its constituents.

Mitochondria were apparently first observed in plant cells, but their first adequate description and suggested function resulted from the study of insect cells. Their ubiquity and apparent homology in plant and animal cells created a new problem for the plant and animal cytologist alike. They have also diverted, to some extent, the attention of cytologists from the nuclear phenomena alone to the protoplast as a whole and have served to emphasize the interrelations between the nucleus, the cytoplasm and its constituents.

Of the many synonyms occurring in the literature, such as "bioblasts, granules, filaments, microsomes, paramita, plasmafaden, plasmakoren, plastosomes, plastokonten, plastochondria, plasmosomes, histomeres, sphaeroblasts, chromidia, chondriokonts, chondriomites, chondriosomes, chromatinosomes, Körner, Stäbchen, Fäden, cytosomes, polioplasma, vibrioden, mitochondria," *etc.*, only the term "mitochondria," coined by Benda in 1897, is retained here, on the grounds of common usage and a certain priority. The collective term "chondriome" is employed in reference to the entire mitochondrial complement of a cell or tissue. The term "mitochondria" refers to those granules, rods, or filaments in the cytoplasm of nearly all cells which are preserved by bichromates within a pH range approximately between 4.6 and 5.0, and which are destroyed by acids or fat solvents.

Archiplasts and proplastids are regarded as identical, and with the plastids collectively comprise the plastidome.

HISTORICAL

There is little agreement as to the identity of the individual who first described mitochondria. Sharp (281) says that they had no single discoverer, and E. V. Cowdry (45) is of the opinion that Flemming, Koelliker and Strasburger must have seen them. Cavers (38) and Meves (202) state that von La Valette St. George (287) in 1886 appears to have been the first to describe mitochondria, whereas Guilliermond (104), Mangenot and Emberger (193)

credit Altmann with their discovery. Lewitsky (184) maintains that Hofmeister had described them in 1851 in *Equisetum* as

Im Äquator der Zelle, zwischen diesen beiden Zellkernen, bildet sich nahe der Zellwand ein Ring oder eine Platte von Protoplasma-körnchen.

Tchistiakoff in 1874 also described this mitochondrial mantle of the division figure of the spore mother cell of *Equisetum*, and Schimper (270) in 1883 similarly described bodies which were apparently mitochondria (figs. 5, 6, 7), but which he called functionless leucoplasts, in the roots and epidermis of *Dahlia*. Bütschli (34, 220), as early as 1861, in his reaction against the concept of protoplasm as a homogeneous body, disputed *a priori* the possibility of fluid protoplasm's being able to carry on the complicated functions of the cell, and later supported his theory by demonstrating granules, which were indubitably mitochondria, in the streaming protoplasm of *Urtica*. Hanstein (34, 223) in 1880 defended Bütschli's view and described the granules observed by Bütschli and himself as microsomes. Martin (34, 192) and Flemming (34, 178) in 1882 and Pfitzner (34, 193) in 1883 also described them, Martin having noted their similarity in appearance with micrococci.

Altmann in 1886 (34, 195-196) first brought forward evidence that the protoplasm of almost all cells contains granules. Under the category of granules he included chlorophyll granules, pigment granules, the granulations of plasma cells, of leucocytes, the granules of pancreas, liver and other gland cells. As early as 1886 he pointed out their analogy to bacteria and described them as the real agents bringing about and carrying on the processes of metabolism.

Von La Valette St. George (287) observed them in the male cells of certain insects, stained *intra vitam* with dahlia and called them "cytomicrosomes." He described them as

zum kleineren Theil aus einzelnen stark lichtbrechenden Körnchen bestehen, zum anderen Theil zu mehr oder minder langen Fädchen aneinander gereiht sind.

In the spermatocytes the cytomicrosomes assembled and formed a cap on the nucleus which St. George called a "Nebenkörper," and which Bütschli renamed "Nebenkern." St. George conceded the priority of Bütschli's observations of these bodies. Henking (34) in 1891 and Toyama in 1894 described similar bodies in certain insect spermatocytes. Bredow (32) in 1891 described colorless

structures, usually elongated, designated by him as embryonic chlorophyll granules which were preserved with OsO_4 and which disappeared upon the application of alcohol, ether and sulphuric acid. He suggested that other workers considered them microsome, which they quite possibly were.

However, the chief value of these early investigations was largely heuristic, for the first detailed observations were not made until 1894, when Meves and Benda demonstrated the presence of mitochondria not only in sex cells of insects but also in the sex and somatic cells of a large number of vertebrates and invertebrates. Benda (15) termed these bodies "mitochondria" (*mitos*, thread; *chondrion*, small grain) because of their tendency to occur in rows or filaments, and suggested that they constitute a new cell organ characteristic of active cells since they occur in sex and somatic cells alike. Benda's special service was the working out of a new technic for their demonstration. He thought they represented a motor organ of the sperm, because in various animals he found that the greater part of the spermatozoon was developed from the mitochondria which formerly were scattered in the spermatogenous cells.

Meves (202) suggested that Benda's "mitochondria" were identical with the "cytomicrosomes" of von La Valette St. George, and demonstrated the untenability of Benda's motor organ view by cutting off the terminal portion of a living salamander sperm, which, though containing no mitochondria, remained motile. Shipley (282) has more recently concluded that mitochondria are not associated with motility in the Trypanosomata.

The first detailed description of mitochondria in plant cells was made by Meves (203) in 1904 who observed them in the tapetum cells of young anthers of *Nymphaea alba*, fixed with Flemming's fluid and stained with haematoxylin. They were considered identical with the mitochondria found in animal cells.

ORIGIN

As to the origin of mitochondria, some of the earliest observers adopted Goldschmidt's (90) "chromidial apparatus" view, *i.e.*, that all active metabolic and other changes in the cell are inaugurated by the extrusion of nuclear chromatin into the cytoplasm, where, either directly by chemical change or indirectly by the provision of energy set free by its decomposition, it assists in bringing about various metabolic processes. He postulated the identity of the chromidia

of protozoa, the chromidial strings of *Ascaris* cells, chondriosomes and plastosomes, the net-like Golgi apparatus, and the centrophormium of Ballowitz with a part of the idiosomes whose common origin was the nucleus. This view was later modified after the appearance of Meves' (203) work. Beer (14) observed bodies similar to those found by Meves in the tapetum cells and stated that they were invariably of nuclear origin, arising either from the chromatin and nucleolar substance or from the peripheral portions of degenerating nuclei. Tischler (295) described them in the tapetum cells of *Ribes intermedium* and von Derschau (63) in various monocotyledons and in *Vicia faba*, and both attributed them to nuclear extrusions. Arnoldi and Börnicke (7) also described mitochondrion-like bodies in several species of plants and considered them nuclear in origin and serving a nutritive function.

Mitochondria in the cells of *Hyacinthus* roots and *Pisum* seedlings were described by von Smirnow (284), who also considered them of nuclear origin. But he said that despite the similarity between plant and animal mitochondria and their staining behavior, nothing definite can be known of their nature so long as microchemical data are lacking. Schiller (38) in 1909 adopted Goldschmidt's chromidial theory and combined it with that of the existence in plants of two independent nuclei corresponding to the meganucleus and micronucleus of certain protozoa, regarding the chromatophores as representing the meganucleus. He could find no trace of plastids in resting embryos of *Triticum* nor *Phaseolus* but thought they arose later from the nucleolus which passed out of the nucleus into the cytoplasm and there broke up into grains which lost their stainability with iron haematoxylin as they developed into chromatophores. Alexieff (2), similarly, ascribed their nuclear origin from the meganucleus of the protozoa *Opalina*, and more recently Riker (256) observing the behavior of mitochondria in *Chara*, concluded their origin to be from the nucleolus. During anaphase of mitosis, prochondriosomes appear on the central plate, after which they migrate out into the cytoplasm and become mitochondria. Motte (220) likewise has connected the extrusion of chromatin with the evolution of the chondriome in the mosses.

Tischler's work (295) with the tapetum cells of *Ribes intermedium* was severely criticized by Duesberg and Hoven (68), who considered that his observations were based upon faulty materials

or methods. They found mitochondria in various plants treated with Benda's method but stated emphatically that these bodies did not arise from the nucleus and therefore could not be considered chromidial in nature. Later Tischler himself (296) in a subsequent review of Pensa's work came to the conclusion that he had, in fact, worked with degenerating tapetum cells and had described phenomena resulting from the death of the nucleus and having nothing to do with chromidial bodies found in meristematic or healthy cells.

Competent investigators have long since abandoned the chromidial apparatus theory and all lingering doubts were adequately removed by Milovidov (217) in a recent paper in which, by the use of the Feulgen technic, he clearly demonstrated the untenability of the theory. That mitochondria do not contain chromatin and have no genetic connection with the nucleus now seems clear.

A few investigators have regarded mitochondria as artefacts produced by the coagulation or precipitation of the cytoplasm by fixation. This view apparently originated with Lundegårdh (188) in 1910, who, as a result of his observations on the root tip cells of *Vicia faba*, concluded that mitochondria were artefacts caused by the action of various fixatives upon the leucoplasts. Schaxel (268) concurred with this view, and Cavers (38) saw a resemblance between mitochondria as described by Meves, Lewitsky, Forenbacher and others and the myelin forms described by Nestler and other writers on the biochemistry of lipoids. Nestler added ammonia to oleic acid and, observing the reaction under the microscope, saw forms like mitochondria appear. Lowschin (185, 186) saw more than an outward resemblance between these, for lecithin products, like mitochondria, can be fixed with chromic acid, osmic acid or formalin, but are destroyed by acetic acid. The analogy, according to Cavers, may go further. Fauré-Fremiet (78) regarded mitochondria as consisting of an albuminoid or protoplasmic ground mass combined, either by ordinary chemical union or by adsorption, with a lipid substance. The fixatives used in mitochondria studies render lipoids in general insoluble in fat solvents such as xylol, alcohol, *etc.*, whereas fatty bodies thus fixed are stained like mitochondria by methods regarded as specific for the latter. The work of Fauré-Fremiet and Lowschin suggested to Cavers that mitochondria may be simply emulsion forms of myelinogenous substances representing plastic food materials.

Kingsbury (172) also doubted the *sui generis* nature of mitochondria. As evidence against it he suggested:

1. The variability of their form in the same or different cells. What is to be regarded as the more primitive form? Meves found rod-shaped mitochondria in chick embryos; Rubaschkin found granules predominant in the embryos of guinea pigs.

2. Their apparent inertness and the indifference with which they are treated at cell division.

3. The unsatisfactory evidence of their mode of formation, *i.e.*, *de novo* or from pre-existing mitochondria, or from chromatin.

4. Conditions of technic. Is every granule a mitochondrion?

Retzius (255) complained of the difficulty of finding out from the literature just what constituted the plastosomen, and criticized Benda's and Meves' work, in which the latter claimed to have demonstrated that his "chondriosomes," Flemming's "fila" and Altmann's "granules" were identical. According to Retzius, the whole idea is problematical and he designated the work as "unreife Forschungsfrüchte" and a "warnendes Beispiel." Dangeard (59) also suggested that the long strand-like, polymorphic figures which stain (and which are considered mitochondria) were only cytoplasmic strands which were ruptured and became chromatic on contact with the vacuoles. They were described by Moreau (219) as evanescent and without individuality.

More recently, Weier (309-311) and Scarth (267) have questioned the *sui generis* nature of mitochondria. The former suggested that the "active" chondriome described by Guilliermond, Parat and others were not mitochondria at all but artefacts produced by fixation. Scarth, from his micrurgical studies on plant protoplasm, was inclined to regard mitochondria as a purely evanescent phenomenon in the cell. Instead of active organs he considered them merely reserve substances for the formation of the labile kinoplasm. This reciprocity of substance was suggested to Scarth by his observations with vital stains. Vital staining increased the mitochondrial elements as the kinoplasm disappeared.

In contrast with these opinions, Seifriz (275) states:

The problem of mitochondria has not to do with their existence but with their role in life. Mitochondria have a persistent identity and perpetuate themselves by multiplication. If reproduction is an infallible criterion of the living state . . . they are living.

The question of the presence of mitochondria in the living cell is no longer a matter for debate, but this problem has been compli-

cated by the fact that the many investigators used a variety of fixatives, a few of which appear to have adequately preserved the chondriome while in other cases artefacts were produced which have led to various misconceptions. This has been adequately demonstrated by Newcomer (226), who compared the fixation images produced by the many fixatives variously recommended by previous workers.

The common criticism of the majority of these fluids seems to be that the different rates of penetration of their constituents result in the over- and under-fixation of the different tissue layers, thus confining observations to a single tissue and preventing a complete picture of the organ. That even the most popular fluids are not immune to this criticism is shown by the fact that N. H. Cowdry (50), using Regaud's method, was forced to confine his observations to the cortical cells of the root tip of *Pisum*. Guilliermond (131) used this method almost exclusively in his work and apparently found it satisfactory, whereas Bowen (25) reported it worthless. Weier (309), working with sporogenous tissues, said:

That I do not possess the complete sporogenetic history of the plastid after fixation with Regaud's fluid was due to the fact that this fluid does not preserve the plastid.

However, the same fluid was apparently satisfactory for plastid preservation in spermatogenous tissues, for he later (310) reported:

Regaud fixation with haematoxylin staining at times also shows a rather striking similarity between the somatic plastid and that found in the androgone.

and concluded that therefore the androgonal body is a plastid. Weier (310) found no mitochondria in the spermatogenous cells of *Polytrichum* and expressed the opinion that the mitochondria Bowen described in these cells were elements of the plastidome and not of the chondriome.

This anomalous behavior of fixatives remained to be at least partially explained by Zirkle (317-322) in a series of excellent investigations of the fixing properties of a wide range of chemicals, from which it seems clear that the fixation image of a given combination of chemicals is hardly less dependent upon the concentrations used than upon the pH of the final solution.

A *de novo* origin of mitochondria has been postulated by several

investigators. R. L. Lewis and W. H. Lewis (178) made extensive investigations of mitochondria in tissue cultures of chick embryos kept living in Locke's solution for several days. Variations in morphology were so great that it was difficult to conceive of them as belonging to the same class of granules, save for their specific staining with Janus green B. The apparent increase and decrease of mitochondria under various conditions were thought to indicate their possible disappearance and *de novo* origin. Rod-shaped mitochondria were seen to change into granules, or into fused or branched threads, and granules fused to form larger granules. Degenerating mitochondria separated into smaller granules and vesicles, an observation also reported by Scott (274). Devisé (64) was of the opinion that mitochondria originated by the fusion of lipid vesicles and droplets of various sizes and Kozlowski (174) postulated a similar origin for the plastids. Orman (234) was uncertain whether the mitochondria in the embryo sac of lily were embryonic plasts, a special form of deutoplasm, or constituent elements of protoplasm, but concluded that they were of cytoplasmic origin, as did Popovici (246).

Horning, in one of his earlier papers (140), reported the presence of mitochondria in all stages of the life cycle of protozoa. Later, however (146), he withdrew from this position, maintaining their disappearance at encystment and subsequent *de novo* origin in the freshly liberated sporozoite stage of the life cycle of *Monocystis*. Horning also reported observation of the division of mitochondria in a living cell and actually timed it. Division occurred by longitudinal or transverse fission and did not synchronize with mitosis.

Causey agreed (35) with Horning as to their *de novo* origin but insisted there was no convincing evidence offered for their division in spite of the fact that Meves, Duesberg, Fauré-Fremiet, the Lewises and others have all reported observing division. Moreau (219) also reported mitochondria as appearing *de novo* and disappearing in the cell and fusing with and separating from protoplasmic substances. Stone (288), investigating the origin of chloroplasts in the potato, found no mitochondria throughout her study.

A large majority of workers and the accumulation of recent evidence do not support the *de novo* theory of origin of mitochondria. Mechanical injury and inadequate technic, of course, seem to suggest it, but it is safe to say that the evidence for the *de novo* origin

of mitochondria is no more convincing than that adduced for the *de novo* origin of the vacuole.

PHYSICAL AND CHEMICAL NATURE

Unfortunately the only available methods for ascertaining the chemical constitution of these bodies are indirect ones which yield, perforce, only approximate data. More specific microchemical analyses are hindered by the fact that the individual components of the complex mask to some extent the identity of the several compounds, and their extreme fragility rather narrowly limits the methods available.

The lipoidal nature of mitochondria was deduced by the early workers from their susceptibility to fat solvents in killing fluids, melting point, *etc.*, and their protein nature from their negative reactions to fat stains, their polymorphism and apparently high degree of hydration and their supposed transformation into plastids which were of known protein and lipoidal structure.

In 1911, Lewitsky (180) published an excellent paper containing the results of his study of mitochondria in fixed and living tissues. He photographed the mitochondria of fixed and living tissues side by side and clearly demonstrated that mitochondria were not artefacts. He reported them as being more light-refractive at times and as changing shape frequently. Küster (176) observed a similar polymorphism in the chromatophores of *Orchis* under the influence of plasmolysing agents. He compared the pseudopodium-like extensions and retractions of the chromatophores with fixation artefacts and concluded that the leucoplasts of *Orchis* were fluid in nature.

A comparison of mitochondria of plant cells with those of animal cells was made by N. H. Cowdry (48, 50), using the radicle of *Pisum* and the acinus cells of the pancreas of the mouse. Both tissues were fixed, dehydrated, cleared, and embedded in the same bottle to secure uniform conditions. Various fixatives were tried and the response of mitochondria to fixatives was identical in both plant and animal cells, *i.e.*, similar fixatives preserved, modified or destroyed them alike. They were also alike in their response to mechanical manipulation before fixation, being extremely fragile and susceptible to destruction. He found no stain specific for them when properly fixed, the same stains exhibiting similar results with both tissues. From these observations he concluded that the mito-

chondria in plants and animals are identical morphologically and microchemically and therefore are composed of precisely the same materials. The physical and chemical similarity, if not identity, of mitochondria in plants and animals is now commonly accepted by most cytologists.

As to their chemical constitution, he admitted the evidence is unsatisfactory inasmuch as no direct chemical analysis can be made and one must rely upon the use of solvents, special stains, *etc.* They gave indications from these methods of containing phosphatids, *i.e.*, they contain fatty acids, phosphoric acids, glycerol and some nitrogen base. The protein constituent of mitochondria assumed by other investigators reacted uncertainly with Millon's reagent. Evidence as to their phosphatid nature in addition to their solubility in fat solvents was deduced from their low melting points, comparable with phosphatids—between 48° and 50° C., when treated for 30 minutes.

Centrifuging gave uncertain results but indicated a specific gravity greater than protoplasm.

Growing plants in a solution of lecithin increased the diameter of the mitochondria considerably, but Cowdry's results were not comparable with those of Russo and van der Stricht, who claimed to have increased the number of mitochondria in oocytes of fowls by lecithin injections. Cowdry (49) later investigated the mitochondria of a number of slime molds and corroborated the results of his previous investigations, with the exception that the mitochondria of these organisms never contained chlorophyll. No indications of a *de novo* origin were observed.

Mangenot and Emberger (193) also compared the mitochondria of plants and animals, using the liver and kidney tissues of the frog and the root tip of the fern *Athyrium*, both of which were fixed and stained by Regaud's method. Their work corroborated in every respect that done by Cowdry, the structures appearing homologous. They made the additional observation that the mitochondria differ from the vacuole-producing bodies, not in appearance but in the fact that the latter do not stain intra-vitaly.

Northen (232) centrifuged the cells of the root tips of *Phaseolus* for 15 minutes at 1000 times gravity and reported the mitochondria as not being thrown down; Jones (150) centrifuged cells of *Elodea* and *Triticum* for 15 minutes at 240,000 times gravity and reported the mitochondria layered between a band of cytoplasm and

osmiophilic platelets. The structures designated in his figures as osmiophilic platelets appear to be mitochondria. My own experiments in centrifuging at 2500 times gravity for any time over 10 minutes have resulted in the total destruction of the chondriome.

Giroud (88, 89) was of the opinion that mitochondria were emulsoid structures, and from observations made upon the filamentous mitochondria of *Ascaris cana* fixed in formalin reported that they exhibited double refraction with polarized light and crossed nicols. If this observation can be confirmed it constitutes the only distinction of importance between plant and animal mitochondria. Newcomer (226), having made numerous observations with polarized light upon mitochondria in plants with living and fixed material, never observed anisotropy of the mitochondria.

Experimenting with various solvents, as acetic acid and ether, Young (315) corroborated the opinions of Regaud, Fauré-Fremiet, N. H. Cowdry, the Lewises and others as to the protein and phospholipin constituents of mitochondria. On very slight evidence, Nicholson (227) reported qualitative chemical differences in the mitochondria of different types of nerve cells in the brains of white mice. His microchemical data were based upon the various resistances in the different cells to the solvent action of acetic acid. This has been a common observation in plant cells but is not to be interpreted as due to qualitative chemical differences. Wen Chao (313) reported mitochondria as giving a positive test for protein with Millon's reagent.

Of the general opinion that mitochondria are a combination of lipids with proteins, Heilbrunn (137) says:

As a matter of fact not only the mitochondria but also the whole mass of the cytoplasm apparently consist of a combination of protein with lipid so that such a chemical constitution can scarcely be regarded as peculiar to mitochondria.

The following table presents a few comparative histochemical reactions of the true fats, phosphatids and mitochondria.

	Millon's reagent	Sudan III Nile blue	Double refrac- tion	OsO ₄	OsO ₄ after K ₂ Cr ₂ O ₇	Solubility in C ₂ H ₅ OH	CHCl ₃
True fats	-	+	-	++	+	+	+
Phosphatids ...	-	-	+	++	-	+	+
Mitochondria ..	+	?	-	?	++	+	+

From the above table it appears that mitochondria may contain compounds of proteins, true fats and phosphatids; the double refractive properties of the phosphatids being possibly masked by the fat and protein constituents. Further evidence as to their phosphatid content is suggested by their reaction with haematoxylin after a dichromate mordant. According to Lee (177), the different fatty substances vary in the readiness with which they are oxidized by dichromates and consequently reach the stage of staining with haematoxylin after different periods of mordanting with dichromates. The lipoids, when pure, differ greatly in the ease with which the stainable compounds are formed: thus, the cerebrosides and protagon stain after a short mordanting, the unsaturated true fats require a slightly longer time, while lecithin is very resistant and requires a prolonged mordanting. Mitochondria in plants require a mordanting of from five to seven days in $K_2Cr_2O_7$ before staining with haematoxylin.

Guilliermond (126) claims to have stained them *in vitro* specifically with Janus green B and dahlia violet. The specificity of Janus green B for mitochondria in animal cells is, I believe, generally accepted. In plant cells, however, its efficacy is sharply debated. E. V. Cowdry (43), Cowdry and Olitsky (47), Marston (195), Sorokin (286), Tarwidowa (291), and others have all reported the specific staining of mitochondria in plants with Janus green. I. W. Bailey (8), from his observations of living cells described mitochondria but was unsuccessful in staining them with Janus green B. He suggested that the vital staining of the chondriome is actually a concomitant of fixation or other irreversible changes in the mitochondria and cytoplasm. Zirkle (316) also stated that Janus green B was not specific for the chondriome. In the protozoa, Hall (133), Causey (37), and Horning and Petrie (147), all report its staining the chondriome, whereas Browne (33) said Janus green B revealed no mitochondria in living material. The author has never observed it staining the chondriome and is of the opinion that the staining reported by these investigators may have been due to its penetration and consequent change of the refractive index of the cytoplasm, thus rendering the mitochondria more sharply outlined against the cytoplasmic background.

Arthur Meyer (212) stained the "Allinante" of *Allium* with neutral red and trypan blue, the latter of which also stained the plastid. He also claimed to have stained the mitochondria of *Poly-*

trichum with neutral red but could not with Janus green. These structures described as "Allinante" and chondriosomes by Meyer (210, 211) were apparently constituents of the vacuome.

In 1933, Bensley and Gersh (18), using a technic heretofore untried specifically for mitochondrial investigations, achieved some new and interesting results. The technic used was a modification of the Altmann freezing-drying method, by which tissues are killed and dehydrated without the aid of chemical reagents. The material used was liver tissues of *Amblystoma*. This material, when sectioned and placed on a slide, was said to have demonstrated that the dried organic basis of mitochondria was not soluble in acetic acid nor in alkalies, alcohol, chloroform, acetone, sulfuric ether, etc. Further, the mitochondrial substance after freezing-drying does not melt at a temperature of 48° to 50° C., but withstands a temperature of 140° C. The main mass of substance is protein in nature, according to Bensley, as indicated by the positive reaction to Millon's reagent. The Millon's reagent used was a modification devised by Bensley of the original formula and is a vast improvement.

The apparent susceptibility of mitochondria in living cells to lipid solvents is not due, concluded Bensley, to the actual dissolution of the mitochondrial substance itself, but is the result of complicated reactions in which the whole cytoplasm participates—an autolytic process of the cell.

In a continuation of the studies on cell structure by the freezing-drying method, Bensley and Hoerr (19, 20) attempted the first qualitative and quantitative analysis of mitochondria. They observed that the mitochondria of the liver of guinea pigs and rabbits were insoluble in an 85 per cent solution of NaCl and water, in both frozen-dried and fresh material. This material, after comminution by kneading or grinding through batting silk and after being centrifuged, was said to have yielded a residue of mitochondria sufficient for chemical analysis. Of this supposedly mitochondrial substance, analysis revealed that 43.6% of the dried weight was soluble in alcohol, ether and chloroform, the distribution of substances for each solvent not having been determined. They concluded that mitochondria contain neither lecithin nor cephalin, but two proteins could be separated by differences in their isoelectric points. In a later paper (17), Bensley gave the following percentage composition of dried mitochondria of the guinea pig liver:

Proteins and unknowns	64.67
Glycerides	28.88
Lecithin, cephalin, <i>etc.</i>	4.2
Cholesterol	2.25

The predominating fats are glycerides, not phospholipins as had been supposed. His conception of a mitochondrion is a body whose organic substances are concentrated at its surface, this surface concentration consisting of a mosaic of protein and various lipoidal micellae and as such he regards them as coazervates. He concluded that "the analyses of mitochondria suggest that the main cortex of the mitochondrial unit is a mosaic of protein, glyceride and cholesterol molecules, rather than a lipoidal membrane."

Observations on a limited amount of plant and animal material killed and dehydrated by a modified Altmann technic (92), placed at the author's disposal by Professors Goodspeed and Uber of the University of California, revealed a chondriome which, with few exceptions, was only indifferently preserved. The mitochondria in this material gave a positive test for protein with Bensley's modification of Millon's reagent, was unstained by any fat-soluble stains but blackened intensely with osmic tetroxide after bichromate treatment. After mordanting from five to seven days in potassium bichromate, they could be stained with haematoxylin.

Observations on mitochondria in living plant tissues were conducted by Price (250) with the aid of dark field illumination. In *Spirogyra*, the mitochondria were observed to have an oscillatory movement as well as one of translocation, gradually passing one another in different directions and giving the appearance of moving in a viscous medium. My own observations on fresh material of leaf and petal epidermis and root-tip sections with dark field illumination and high magnification revealed a multitude of flickering, light-diffracting bodies scattered throughout the cytoplasm, exhibiting every degree of excitation from Brownian movement to a rather rapid movement over the field.

Strangeways and Canti (289) also observed mitochondria in living cells and reported them as showing a writhing, snake-like movement in the cytoplasm. In poorly growing cultures the mitochondria were seen to thicken and appear as oval or club-shaped bodies which tended to fragment. This change from rod-shaped to spherical form was also observed by Tiegs (294), who attributed it to a physical alteration from the solid or highly viscous condi-

tion of the rods to the liquid condition of the globules. This fragmenting of filamentous mitochondria into shorter, thicker chainlets and granules was also observed by Weatherford (308), who attributed it to their sensitivity to cellular injury.

The mitochondria of animal cells growing in a tissue culture were investigated by Smith (285) and Fischer (80) and described by the latter as extremely plastic bodies which react more rapidly than any other cell structure to such stimuli as changes in the osmotic pressure of the surrounding medium, heat and chemicals.

In 1914, R. L. Lewis and W. H. Lewis (178) made extensive investigations of mitochondria in tissue cultures of chick embryos kept living in Locke's solution for several days. They tried various killing fluids with the material under observation and corroborated previous workers on the subject as to their action. Acids destroyed them, whereas osmium tetroxide, potassium bichromate, formalin, *etc.*, preserved them. They found great variation in size, number, position and morphology of the mitochondria in all cells. Variation was so great that it was difficult to conceive of them as belonging to the same class of granules, save for their specific staining with Janus green B. The mitochondria were counted and no one number found peculiar to any cell or type of cell, contrary to Barratt's (10) earlier findings, in which he reported approximately 70 mitochondria per cell, variations from this number being inconsiderable.

The apparent increase and decrease of mitochondria under various conditions was thought to indicate their possible disappearance and *de novo* origin. Rod-shaped mitochondria were seen to change into granules, or into fused or branched threads, and granules fused to form larger granules. Degenerating mitochondria separated into smaller granules and vesicles, an observation also made by Scott (274). They could not corroborate Meves' and Duesberg's observation on the division of mitochondria, no division having been observed.

Milovidov (214, 216) found the mitochondria in plant cells to be less sensitive than the nucleus to the influence of radium emanations, whereas Nadson and Rochlin (223) reported the mitochondria to be the most sensitive of the cell constituents to x-rays. The first effect was the production of fragmentation forms changing rapidly to polymorphic shapes followed by a final vesiculation

and dissociation of the lipo-proteid complex, the lipoids of which stained with Sudan III and blackened with osmic tetroxide.

As to the physical and chemical structure of mitochondria, one may say that they are apparently highly hydrated, polymorphic rods and granules of a lipo-proteid complex with a specific gravity perhaps slightly greater than that of the cytoplasm and that they are extremely fragile and susceptible to external stimuli. The mitochondria of plants and animals seem to be identical in these respects and are therefore usually regarded as homologous structures.

FUNCTION

Plastid formation

In July and September of 1910, Pensa (237), after studying the ovaries of various plants and carefully tracing the behavior of the mitochondria and nuclei in successive stages of development, concluded that mitochondria gave rise to chromatophores and noted their similarity to the mitochondria of animal cells. He later stated (238):

. . . j'ai été le premier à décrire la dérivation des chloroplasts de formations très fines, semblables par leurs caractères morphologiques et microchimiques aux mitochondries des cellules animales.

Duesberg (67) suggested that Pensa's use of Cajal's silver method was unfortunate and that his figures represent not chloroplasts but artefacts. Pensa denied the implication.

In December of the same year, Lewitsky (179) independently came to the same conclusion and reported:

In der Stengelspitze des Keimlings wandeln sich beidem untersuchtem Objekte die Chondriosomen zu Chloroplasten um, in der Wurzelspitze zu Leucoplasten.

This classic observation immediately aroused a storm of protest and precipitated a controversy which is still undecided. Meyer (210) immediately replied that:

der Satz von Lewitsky, dass die Chromatophoren aus Chondriosomen hervorgehen, ist wohl sicher unrichtig.

Meyer presented no new evidence but quoted from his work of 1883 (209) and from that of Schimper in 1885 (271) as having established the fact that the chromatophores arose only through the division of pre-existing chromatophores. He concluded that there

was no need of his re-examination of the subject until Lewitsky produced more evidence. This Lewitsky did in 1911 (181) by producing excellent photographs of plastid formation in living and fixed cells of *Elodea*. He tried various fixatives and divided them into two groups:

1. Those which gave the real structure of cytoplasm and showed the mitochondria in their natural condition (Benda's fluid; the same minus acetic acid; 5 per cent osmic acid; 10 per cent formalin; weak Flemming).

2. Those which gave deformed products and artefacts (absolute alcohol; Carnoy's acetic alcohol; mixtures containing corrosive sublimate; silver nitrate plus pyrogalllic acid; strong Flemming).

To the young, forming chloroplasts he gave the term "chondriokont stage."

Forenbacher (81) in 1911 studied the origin of chloroplasts and leucoplasts in the root and stem of *Tradescantia* and came to the same conclusions as Lewitsky and Pensa. For fixing he used a modified form of Benda's fluid, acetic acid omitted, and stained with Meves' haematoxylin and the triple stain of safranin, gentian violet and orange G.

During 1911-1912, Guilliermond (95-103) published a series of papers with special reference to mitochondrial function, confirming and extending the work of Lewitsky, Pensa and Forenbacher. He examined plants belonging to all the main divisions of the vegetable kingdom and found mitochondria in nearly every case. In germinating seeds of grasses he found mitochondria in the cotyledons mostly unchanged, whereas those in meristem cells disappeared as plastids were formed at their expense. They were equally distributed to the daughter cells during mitosis, after which they segmented to form short rods; with leaf development they swelled and were transformed into chloroplasts.

In 1912 Guilliermond (104) summarized his own and others' work on the origin of plastids from mitochondria and stated that the origin of plastids does not afford a sufficiently exact definition of the function of mitochondria. Hence he tried to define and to distinguish these bodies by their microchemical characters and from his results concluded that, although plastids show the same stain reactions as mitochondria, the latter are usually soluble in alcohol and acetic acid, whereas the former are not.

The observations of Pensa, Lewitsky, Forenbacher, *et al.*, regard-

ing the mitochondrial origin of plastids were corroborated by Maximow (196) from his observations on the living cells of *Cucurbita*. Twiss (298), Faull (77), Newcomer (226) and others have also demonstrated all the transition stages from mitochondria to plastids in the root tips of *Zea mays*.

Basing his conclusions upon his investigations of the origin of chloroplasts in the cotyledons of *Helianthus*, Miller (213), in 1911, denied their mitochondrial origin and maintained the presence of chloroplasts in the resting seed. These alone, he asserted, gave rise to those of the mature plant. The chloroplasts in the resting seed were very minute, according to Miller, but with the onset of germination they increased in size and began to divide by fission. Miller's use of chromacetic acid for fixation was unfortunate. In this he followed Famintzin, whose conclusions he corroborated.

Meyer (210) also criticized the findings of Lewitsky, Pensa, Forenbacher and Guilliermond, regarding the origin of chromatophores of flowering plants, maintaining that chromatophores always arise from pre-existing chromatophores and not by a morphological and chemical process of transformation from mitochondria. He demanded more definite proofs that mitochondria can be distinguished from small chromatophores and that the actual transformation of the former can be seen directly in the living cells of filamentous algae.

In 1912, Schmidt (272) published a general review of the work up to that year. Cavers (38) considered the review incomplete and misleading, distorting the results to agree with the Schimper-Meyer view that chromatophores always arise from pre-existing chromatophores. Schmidt concluded that these recent workers were dealing simply with the very early stages of the development of plastids which their technic enabled them to observe and which the simpler methods used by Schimper and Meyer were inadequate to demonstrate. According to Schmidt there are three possible interpretations of plant mitochondria. These bodies are either:

1. Chromidia corresponding to those of animals, *i.e.*, portions of chromatic material derived from the nucleus, or
2. Chromatophores in early stages of growth, or
3. Special cytoplasmic structures.

With regard to the first, he said that few botanists believe that plant mitochondria are actually extruded nuclear bodies, and this view can be accepted only when confirmed by observations on living

material; as to the second, that chromatophores in all stages of development give the same staining reaction as those considered characteristic for mitochondria and in his opinion the new workers have simply brought good support to the Schimper-Meyer view. As to the third view, he denied altogether its probability. Schmidt (273) later expressed the opinion that the bodies described as mitochondria were variable forms of chromatophores in different stages of development.

Using the shoot tips of *Asparagus officinalis*, Rudolph (259) investigated the relationship of chloroplasts to mitochondria. His observations agreed with Lewitsky's on the whole, as regarding the mitochondria themselves, but he could find no relation between these and the chloroplasts. He found granular bodies in the youngest cells, later—or farther back—filamentous ones, evidently elongations of the granules prior to their division. Some granules of young cells grew rapidly and gave rise to chromatophores; others remained at their original size. He tried to draw a sharp distinction between the two kinds and said that chromatophores and mitochondria are of entirely different natures and there is no genetic connection between them. He admitted he could find no transition forms as described by Pensa, Lewitsky, Forenbacher and Guillemont, but could detect no difference between the granules which gave rise to chromatophores and those which gave rise to mitochondria found in older cells; yet he concluded that those granules are of different natures and supported the Schimper-Meyer view of the origin of the chromatophores.

This reasoning is borne out by the argument that if Lewitsky's view be adopted we have a sharp break in the phylogenetic series. It is admitted that in algae with a single large chromatophore, the latter is always derived from that of the parent cell or coenocyte, while according to Sapehin (262) all the chloroplasts in a moss plant are derived by division from the single chromatophore present in the spore; hence it is *prima facie* improbable that the chromatophores of higher plants should originate in a different manner.

Sapehin found complete genetic continuity of the plastids in the Pteridophytes *Lycopodium*, *Selaginella*, *Isoetes*, also in *Funaria* and *Anthoceros* of the Bryophytes. Later (263, 264) he found mitochondria in all the mosses investigated (species of *Polytrichum*, *Funaria*, *Bryum*, and *Mnium*). They occurred in nearly every cell of the gametophyte and sporophyte and were abundant in the pro-

tonema, apical cells of stem, embryo, germ cells and spore. However, plastids occurred side by side with them and apparently they were unrelated.

Scherrer (269) and Newcomer (226) also found no relation between mitochondria and chloroplasts in *Anthoceros punctatus*. Weier (309) likewise found them unrelated in *Polytrichum commune*. The latter reported:

From the appearance in the author's preparations, he is inclined to support the theory of Meyer that the mitochondria are not definite living cellular structures, but some stage in the development of cellular products.

Randolph (252) concluded his investigations on the chlorophyll types of *Zea mays* with the statement that:

The division of young and mature plastids emphasizes the fact that they have a distinct individuality at such stages, but in view of the obscurity which surrounds the origin of the minute primordia from which the plastids first appearing in the embryonic cells arise, the question regarding the extent to which the plastids are to be considered permanent cell organs with an unbroken genetic continuity throughout the life cycle must remain an open one.

Barton-Wright (11) concurs in this uncertainty of the genetic continuity of the plastid throughout the life cycle of the higher plants.

In 1919 Harper (134) said of the investigations to date that:

None of the evidence so far adduced as to a specific genetic relationship between mitochondria and plastids is in any way adequate. There is an equivalence in the weight of testimony in the literature on both sides of the question. That granules, rods, strands, etc., can be observed in the cytoplasm is undoubted and has long been known. The claim that those taking a certain stain after a given fixation can all be classed together as coordinate unit elements, while suggestive, needs further confirmatory proof like that which has been accumulated for the individuality of the chromosomes.

E. V. Cowdry (46) in 1926 wrote:

That we are faced by a process of differentiation whereby the mitochondria, with the assistance of the cytoplasm, change into larger and structurally more complex plastids is highly probable. Yet it has been questioned on the supposition that the bodies referred to as mitochondria may, in reality, comprise, in addition to true mitochondria, other fundamentally different structures, which constitute the actual primordia or *anlagen* from which the definitive

structures are formed. It is difficult to rationalize this attitude of mind. . . . It becomes less justifiable as the years go by, and investigators become more and more proficient in the identification of mitochondria. . . . That mitochondria and plastids are related genetically seems clear.

Zirkle (316), investigating the origin of plastids in *Lunularia*, *Elodea* and *Zea mays*, observed that the epidermal cells of the mature leaf of *Zea mays* contain neither plastids nor primordia, in spite of the fact that the dermatogen is rich in primordia. On the other hand, they contain many mitochondria which can be traced back to the growing point and seem to be merely undeveloped or degenerate plastid primordia. He further observed a difference in the plastid development of the stem and root. In the stem growing point the primordia are essentially tiny plastids, but in the root tip they cannot be distinguished from mitochondria. This he explained by making two assumptions:

1. The plastid primordia may be able to reproduce by division during several different stages of their development into plastids. Such divisions have been reported by other workers even in mature plastids.
2. It is the reproduction rather than the growth of the primordia which varies with the rapidity of cell division, since in this case we should expect smaller primordia when cell division is more rapid, as in the root meristem.

Finally he stated that primordia do not arise *de novo* but are reproduced from pre-existing primordia by division, which occurs in one plane only. Their division in one plane only is deduced from their frequent occurrence in chains and never in plates. Zirkle further stated that Janus green B is not specific for mitochondria in plant cells, as reported by Cowdry and others.

In a later paper, Zirkle (317) confirmed his previous findings regarding mitochondria and plastid primordia, stating that these two categories are but developmental stages of a single cell organ; the fact that either may occur in a given tissue indicates that plastid development is to a certain extent independent of the plant as a whole. Recent evidence seems to indicate the essential correctness of this point of view.

Senjaninova (277, 278), a student of Lewitsky, having investigated the origin of plastids during sporogenesis in mosses, concluded:

Thus the investigation of the mosses in the critical moment of their existence, on the border of two generations (sporophyte and gametophyte) has shown that the plastid of the moss is nothing but a cell chondriome differentiated in a special way.

The complex plastids of mosses originate not by means of the transformation of separate chondriomes, as is the case usually in the higher plants, but through a peculiar condensation of the chondriome.

Recent attempts to trace the chondriome and plastids through the changes involved in antherozoid formation of Bryophytes and Pteridophytes have failed to yield harmonious results. It has been mentioned that Sapehin (264) and Scherrer (269) both maintained that the continuity of the chromatophore throughout the life cycle is clear and both reported the presence of mitochondria which were apparently without morphological connection. The plastids of Sapehin were apparently the centrosomes and blepharoplasts of other writers. Sharp (279, 280) considered the blepharoplast derived from the centrosomes and Chalaud (39) mentioned the regression of plastids into rod-like structures (chondriokonts) before they finally disappeared in the spermatogenous tissue of *Fossombronina*. Weier (309) and Gavaudan (87) found no relation between plastids and mitochondria in the antheridia of Bryophytes. The former regarded the limosphere of plastid origin and never observed mitochondria in the androcytes of *Polytrichum*. Motte (220) regarded the Nebenkörper, limosphere, blepharoplast, plastid, etc., in the androcytes of mosses and hepatics as the condensed chondriome, but Gavaudan denied the transformation of plastid into chondriome and was of the opinion that the cytoplasmic inclusions derived from plastids and interpreted as mitochondria by Chalaud and Motte were in reality metabolic substances. Nevins (224) reported that a few osmic acid preparations of the antheridia of *Sphaerocarpus* seemed to suggest a possible relationship between the plastids and chondriome in young antheridia.

In his earlier researches, Guilliermond (109) could find no chondriome in the algae and fungi, but observing the anastomosed stroma of the chromatophores of *Spirogyra*, *Cosmarium* and *Oedogonium*, concluded that the entire cell chondriome united or condensed to form the chromatophore. Mangelot (192) also reported the chromatophores of *Draparnaldia* as being formed by a confluence of the chondriome, and Kozłowski (175) and Senjaninova

(277, 278) are apparently still of this opinion. Guilliermond (123) later observed mitochondria in the algae by sectioning material 1 micron in thickness. He admitted that it was difficult to homologize the algal plastid with mitochondria but suggested that perhaps it was like the Nebenkern of animal spermatozooids, which is the animal chondriome condensed into a single organ. He said that it was also difficult to homologize the algal plastid with the plastids of higher plants, yet this is unquestionable. In 1914 (108) he published a castigation of the works of A. Meyer, Lundegårdh, Lowschin, Schmidt, Rudolph, Sapehin and Scherrer. He also denied Meves' heredity function of mitochondria, stating that the chief function of mitochondria was secretory. The anthocyan pigments, chlorophyll, carotin, *etc.*, of phanerogams, and the "metachromatic bodies" of fungi are the direct product or expression of living mitochondria. As a concession to Sapehin and Scherrer, he admitted that there might be two kinds of mitochondria in the liverworts, one giving rise to the chloroplasts, the other type secreting. He was also of the opinion that Schimper and A. Meyer's so-called leucoplasts were nothing more than mitochondria. Meves (206) differed with Guilliermond and was of the opinion that the granules described by Schimper were not mitochondrial but metaplastic.

General support to Guilliermond's criticisms of the Schimper-Meyer school was given by Meves (205, 206) in an extensive review in which he tentatively suggested the duality of the chondriome. In various plant cells he distinguished a chondriome like that of animals, *i.e.*, "grains of metaplast" which stained more lightly than chondriokonts—from which he concluded that they were separate; plastids arising only from the chondriokonts; and that it was impossible to say where the chondriokont ended and the plastid began. He called A. Meyer's work false and accused him of wilful assumption and a stubborn adherence to his theory in the face of facts. According to Meves, Meyer was not only wrong in his "Allinante" (211), which were ergastic structures and totally unrelated to the chondriome, but also in his quotations, dates and interpretations of other authors' work. He further described Sapehin's and Scherrer's work as false.

Guilliermond (119) and Guilliermond and Mangenot (130) in a review of some of the literature from 1910 to 1925 gave three theories of the relation of mitochondria to plastids:

1. The plastids are derived by the differentiation of a certain number of mitochondria in embryonic cells. This theory, again supported by Alvarado (however, Alvarado (4) saw no distinction in the origin of chloroplasts of the mosses and higher plants) conforms to facts observed in phanerogams but not with those observed in the algae, Bryophytes and Pteridophytes.

2. The plastids maintain their individuality in the course of development and there is no relation between them and mitochondria. They are different chemically and morphologically. This satisfies the conditions in the lower plants but not in the phanerogams.

3. There exist in chlorophyllous plants two categories of organs, both of which maintain their individuality in development, are transmitted by division, and present the appearance of mitochondria. The one corresponds to the special plastids of green plants, the other is comparable to the mitochondria of fungi and animals.

They concluded:

Cette dernière opinion soutenue par Guilliermond, Emberger, Mangenot et Mottier est la seule qui suit d'accord avec tous les faits: elle est absolument démontrée.

It would be premature to say that this conclusion will meet with unanimous acceptance.

Extensive investigations of plant mitochondria were conducted by Mottier (221) in 1918, using root tips of *Pisum sativum* and *Zea mays*, thalli of *Marchantia*, *Anthoceros*, *Pallavicinia*, the seedling of *Pinus banksiana*, the stem and leaves of *Elodea canadensis*, certain algae and root tips of *Adiantum pedatum*. Of the last named he said that there are two distinct organs in the cytoplasm: primordia of plastids and other bodies which he designated as chondriosomes. The latter vary in shape from spherical granules to short rods, the former always exceeding the latter in number. They divide rapidly with the growth of the cell, becoming very numerous, especially in the large, rapidly elongating cells of the central cylinder. The primordia of leucoplasts, on the contrary, develop in the root cap into bodies resembling certain chloroplasts, which contain one or more lenticular inclusions of starch.

In general, he concluded, it is more probable that mitochondria and chloroplasts, though alike morphologically and in staining properties, are structures of a different nature without any genetic connection between them and in no cases were mitochondria changed into leucoplasts. He was convinced that both primordia and mitochondria are cell organs *sui generis*, of the same rank as

the nucleus, being transmitted from cell to cell by the cytoplasm of the gametes, thus depriving the nucleus of a monopoly in heredity.

Replying to Mottier's criticism of the theory of the mitochondrial origin of plastids, Guilliermond (110-118) stated that he believed it had been demonstrated that pigment bodies in animal cells are formed from mitochondria and hence, by analogy, that the plastids in plants must arise in the same manner. He regarded Mottier's interpretation as based on a lack of recognition of the occurrence of two kinds of mitochondria. Amyloplasts were observed by Guilliermond to arise from rod-shaped mitochondria in *Cucurbita*, whereas in the potato, granular mitochondria may produce them, the two types being thus genetically related.

Smith (285), investigating the relation of mitochondria to pigment formation in cells of the pigment layer of the retina of the chick embryo by the Locke-Lewis method, concluded that mitochondria have nothing to do with pigment formation. His findings were precisely contrary to the supposition made by Guilliermond, and if true destroy the latter's analogy of the origin of plastids, which is based upon the formation of pigment cells from mitochondria in animal cells. Smith's results were later corroborated by Fischer (80), who also used a tissue culture technic.

Supporting the Schimper-Meyer theory, Noack (230) claimed to have demonstrated the distinction between plastids in chemistry, morphology and physiological function. He asserted that Lewitsky's error could be explained by the fact that he had destroyed the mitochondria by inadequate fixation and that Mottier was incorrect because he confused plastid primordia with mitochondria. The plastids in meristems, according to Noack, are identical with mature plastids and function as such, but are smaller in form. Further support to this view was accorded by Nihoul (229) and Sorokin (286). The latter conceded the *sui generis* nature of both plastids and mitochondria but claimed that the two categories of bodies, though morphologically similar, exhibit detectable differences in size and form and may be distinguished in living cells by their differential staining in Janus green B. Emberger (71) also observed two types of mitochondria in the root tips of *Athyrium* which differed slightly in intensity of staining reaction and in size. The darkly staining type gave rise to plastids, but the function of the other type could not be determined.

In 1920, Emberger (72, 73), investigating the development of

the chondriome in the formation of sporangia of ferns, made some interesting observations. In the young sporangium of *Scolopendrium* and *Asplenium* he found lenticular and rod-shaped chloroplasts, chondriokonts, and granular mitochondria. In the spore mother cells the chloroplasts underwent transformation into chondriokonts which stained more deeply in later stages. Chondriomites (granular) were also present at this stage. The chondriokonts dissociated into mitochondrial granules before the reduction division began and these persisted throughout the divisions as granular mitochondria. In the spore they gave rise to the chloroplasts and mitochondrial bodies of various forms. There thus occurs, according to Emberger, a plastid reversion during spore formation.

Friedrichs (82) suggested a similar plastid reversion in seed plants and Chalaud (39) described it in the spermatogenous tissues of *Fossombronia*. Emberger (74, 75, 76) described the process in the higher plants in detail. First the elaborated products, such as starch and pigments, disappear; then the substratum becomes very plastic, irregular and elongated, and alternately contracts and breaks at numerous points. These phenomena succeed each other rapidly and are often accompanied by a cellular rejuvenescence. He also described (76) the reverse process of mitochondrial transformation into amyloplasts and chromoplasts and agreed with Guilliermond that there were two types of mitochondria, designating those which correspond to Guilliermond's "inactive" chondriome as the mitochondria "ordinaires." Seven criteria by which the two types of mitochondria may be separated were given.

The morphology of mitochondria is determined, according to Emberger, by the cellular metabolism or physiological state of the cell. Thus, in areas of heterotrophic metabolism, the plasts are mitochondrial in form; with autotrophy come synthesis and plasts. The only difference between the chondriome of the fungi and algae is that the former never become plasts because of a heterotrophic metabolism. As for some plants (potato tubers, etc.) where the amyloplasts cannot become green, and in others where metabolism is always heterotrophic (saprophytic and parasitic phanerogams), they merely contain a fungi chondriome.

A similar reversibility of the chloroplasts in spore formation was reported by Mangenot (191). In the Lemnaceae the rhodoplasts are elongated, the smaller resembling chondriokonts but containing the pigment material. He found these small rhodoplasts in the car-

pogonium, but after fertilization they were observed to fragment into mitochondrial structures which persisted until the formation of the young carpospores, when they enlarged and the pigment reappeared in the mature spore. Another type of mitochondrion was observed to remain unchanged throughout the life history of the algae.

Ruhland and Wetzel (260) examined pollen tubes grown on culture media of 50 species of plants with a fluorescence microscope and found only three species (*Lupinus luteus*, *Narcissus incomparabilis*, *Crocus vernus*) which contained chlorophyll. These chlorophyll-containing bodies were near the limit of visibility, averaging only .2 to .3 of 1 micron in diameter. Mitochondria were also present, and they doubted their genetic connection.

In 1919, Guilliermond (111) made another investigation into the origin of chromoplasts and the method of formation of the pigments carotin and xanthophyll in plants. He described the cytoplasm as of a colloidal nature, filled with mitochondria in the form of granules, short rods and elongated, sometimes branched chondriokonts, these elements being formed only by division of preexisting mitochondria. They are protoplasmic in nature and play an important physiological role, concluded Guilliermond, since through them are certain products elaborated. In older cells the chondriokonts (rods) increase in size and become plastids. The formation of carotin and xanthophyll was associated with the mitochondria and the plastids derived from them, the pigments occurring within these bodies in the form of minute granules or crystals or, in some chromoplasts, in a diffuse state.

Tchang (293) reported a mitochondrial origin of plastids and described starch grains as having a mitochondrial cortex which, after the starch is resorbed, may develop into a chloroplast.

Kassmann (171) conceded the essential correctness of Pensa's, Lewitsky's *etc.*, view as to the mitochondrial origin of plastids in a cytological study with fresh material. She could not see a direct transition from mitochondrion to plastid, even with continued observation, because of a turbidity in the cells obscuring the appearance of the chloroplast, but reported that no mitochondria were present in the cell after chloroplasts were formed. She further observed that Guilliermond never followed the development of a single individual mitochondrion into a plast but based his results on a composite picture.

Guilliermond (114, 128, 131) attempted to distinguish two categories of elements in the chondriome which he designated as the "active" and "inactive" chondriome. The former elaborated starch and chlorophyll and became plastids, whereas the latter retained their original morphology and apparently remained inactive. This inactive chondriome in flowering plants was considered homologous with the chondriome of fungi, which in turn he homologized with the chondriome of animals. Of the flowering plants, he said, "in most cases it is absolutely impossible to distinguish in meristematic cells, which are destined to become plastids and which remain inactive."

This question of the relationship between mitochondria and plastids was considered by Kozlowsky (175), who differed with Guilliermond as to the precise method by which the transformation from mitochondria to plastids was accomplished. Guilliermond maintained that this was effected by the increase in size and swelling of the mitochondria, but Kozlowsky contended that it was not by swelling of the individual but by the agglomeration and coalescence of numerous, discrete, granular bodies. He made the further observation that mitochondria do not divide nor do they move autonomously in the cell.

In a series of papers between 1927 and 1929, Bowen (23-28) described and defined five cytoplasmic components of plant cells: plastidome, pseudochondriome, osmiophilic platelets, vacuome and oil droplets. The plastidome and pseudochondriome, which correspond loosely to the active and inactive chondriome, respectively, of Guilliermond, are, according to Bowen, functionally distinct. This, Bowen contended, is demonstrated by their differences in staining behavior, morphology, structure and behavior during cell division. In the plastidome, the constituents of which he called "archiplasts," staining with vital dyes is noticeably slower than in the pseudochondriome. Polymorphism is a property of the plastidome, and the constituents of the plastidome are never granular in structure, in contrast to the pseudochondriome. The peculiar radial orientation of the plastidome at the polar caps during mitosis and the absence of pseudochondriome in root-cap cells constitute an added criterion for their functional differentiation, according to Bowen.

Their distinction was most clearly shown in the Thallophyta, Bryophyta and Pteridophyta. Bowen demonstrated the perma-

nence in shape and numbers of plastids in all phases of the life cycle of the mosses, algae and ferns, but found associated with the plastids small spherical bodies (as did Sapehin, 262), which constituted what he called the "pseudochondriome." The plastidome in flowering plants, Bowen stated, has lost its clear-cut individuality because of its failure to maintain a chlorophyll condition throughout the life cycle. The pseudochondriome, on the other hand, has retained its permanence of shape from unicellular organisms through all the vicissitudes of plant evolution and constitutes the homologue in plants of the chondriome in animals. Bowen (26) said:

To my own mind, the distinction which Guilliermond insists upon seems largely a matter of words. The microchemical basis upon which he so clearly relies for the proof of his duplex-chondriome theory, I have shown to be without any critical foundation in staining behavior. . . . Nothing can possibly be gained at present by insisting on its retention, and there would be some advantage in unifying the point of view of all workers who hold that plastidome and pseudochondriome are genetically and materially distinct.

Guilliermond (125) and Reed and Dufrenoy (254) were of the opinion that the osmiophilic platelets described by Bowen were merely mitochondria or vesiculated plastids artefacted by the use of osmic acid, although they presented no evidence to support the opinion. But Bowen maintained the individuality of platelets in the cell by the fact that they are demonstrated only by the osmic impregnation method of Kolatchev and furthermore that they are not vesicular but ring-shaped. The division of plant cell inclusions into five categories described by Bowen was supported by the subsequent work of Patten, Scott and Gatenby (236) and Jones (150).

Beams and King (12, 13), having centrifuged the root tips of *Phaseolus* and hepatic cells of the rat, reported the plastidome and pseudochondriome elements going to the centrifugal pole and the osmiophilic platelets to the centripetal pole, and in general accepted the terminology of Bowen.

Contrary to the findings of Bowen and others, Kirby (173) reported the absence of mitochondria in the very young stages of the sporangia of *Osmunda*. Either, she said, they arise *de novo*, or if present are below the size of visibility. In any case, the *sui generis* nature of mitochondria cannot be established until their persistence throughout the life cycle has been proved. The plastid origin

from mitochondria is clearly shown and the plastid primordia are indistinguishable from the chondriome, according to Kirby. Continuing, she suggested that since there is no obvious difference between granules which develop into plastids and those which do not, it is reasonable to assume that there may be no structural difference. To explain the developmental differences of similar granules, she postulated the presence of a mathematical plan in granule distribution subject to nuclear control, that is, due to the presence of some factor in the chromosomes.

In 1920, Dangeard (55) suggested discarding the terms "mitochondria, chondriosome, chondriokont and chondriomite," etc., and substituting the terms "vacuome" (metachromes and metachromatic corpuscles), "plastidome" (metaplasts and plastids), "spheromes" (microsomes), and "fibrils of the cytoplasm," which he considered to have a more precise significance. All these structures have been usually regarded as mitochondria by other workers.

In *Selaginella* Dangeard described the plastidome, vacuome and spherome as all staining black with haematoxylin after Regaud's fixation. The chloroplasts arise from a small band lying appressed to the nuclear membrane, which stains deeply and which divides just prior to cell division. This band, or mitoplast, gives rise by its division to chloroplasts and is found in meristematic tissues, young leaves, stem cortex, vascular tissue and primordia of sporangia.

In the vacuoles are metachromatic corpuscles which compose the vacuome and which stain as does the mitoplast. As the vacuoles fuse in maturation, the vacuomes may remain single or be grouped into chains or ribbons. They always remain within the vacuole, although the vacuolar membrane may not always be clearly distinguished.

The spheromes are the ordinary microsomes which are isolated or occur in rows or chains, but which are never found in the vacuoles. He further suggested that the mitochondria as described in animal cells were nothing but the first stages of the vacuome and that metachromatin and mitochondrial substances were identical. Furthermore, the long strand-like, polymorphic figures which stain (and which are regarded as mitochondria) are only cytoplasmic strands which are ruptured and become chromatic on contact with the vacuoles. Dangeard (57, 59) further divided the plastidome by morphology into spheroplasts, mitoplasts and discoplasts; and

by function into xanthoplasts, carotinoplasts, chloroplasts, amyloplasts, elaioplasts, etc.

The microsomes of the sperome which were normally spherical he called "spherosomes"; those assuming a batonnet form were interpreted as being deformations or division stages and were named "mitosomes." He later (60) stated that because Guilliermond persisted in confusing his spheromes with common lipoid granules, he was led to substitute the term "cytome" for "spherome."

In 1920, Guilliermond (116) described bodies of mitochondrial form in leaves of *Iris germanica* which became transformed into vacuoles. Disagreeing with Dangeard, he maintained that these bodies were not metachromatic in nature and that they differed in their microchemical reactions from the mitochondria of animal cells. However, he reiterated that there were two types of mitochondria in plant cells (114): one assimilates starch in young leaves and forms plastids; the other is of a non-assimilating nature. In addition to these, there are small globules, probably of a lipoidal nature, which have nothing in common with mitochondria.

Guilliermond was criticized by Dangeard (56) for including under the term "mitochondria" all cell elements giving the mitochondrial reaction, regardless of their origin or development. Dangeard contended that anthocyan and tannins are formed from the metachromatic bodies of the vacuome, Guilliermond maintaining the mitochondrial origin of anthocyanin.

In a later review, Dangeard (61), discussing the application of his terminology to the fungi, described an additional category of cytoplasmic inclusions, the ergastome. This ergastome was comprised of liposomes which arose *de novo* in the cell. He further limited the term "chondriome" to include only the initial stages of the vacuolar system and abandoned (54) the earlier term "chondriosome" which he had used provisionally for these structures. He accused Guilliermond of lumping the plastids, mitochondria and the vacuolar initials into one category, the chondriome. Of Guilliermond's terminology and his division of the chondriome into active and inactive constituents, he said:

Il est donc nécessaire, du point de vue purement scientifique et moral, d'en dégager les tendances et, disons le mot, les sophismes.

Gavaudan (87) accepted Dangeard's terminology and subdivided the ergastome into the mobile or active ergastome and the

immobile or inactive ergastome. The active ergastome was comprised of the usual osmiophilic inclusions of the cytoplasm and was a permanent cell constituent. The inactive ergastome consisted of the oleocarps or fat bodies described by other authors and corresponded to the tentatively suggested adipome of Dangeard. They were reserve secretion products and evanescent in the cell. He further reported that there was no homogeneous chondriome in the hepatics; the mitochondria described in them by others were merely metabolic substances and part of the active ergastome. Gavaudan's drawings of the ergastome in *Madotheca* are identical with the structures depicted by Guilliermond in *Saprolegnia* and described as mitochondria. Of Bowen's osmiophilic platelets he considered it impossible to admit that fixation and impregnation could reveal a structure invisible in fresh material where one never sees anisotropic, refringent globules. However, he used not the Kolatchev technic as prescribed by Bowen, but Flemming's fixation followed by osmium tetroxide.

Guilliermond (115, 121, 124) criticized Dangeard's findings as based upon inadequate observations without the use of a mitochondrial technic. Dangeard confused the vacuome with chondriome, which, though superficially similar in morphology, fix differently and react differently with vital stains. Guilliermond later (129) observed that:

La connaissance du chondriome des cellules végétales a été exclusivement débrouillée par nos travaux et les recherches de M. Dangeard n'y ont pas apporté le moindre éclaircissement.

To which Dangeard replied that he regarded the work of Guilliermond as "l'édifice construit à grand frais . . . s'écrouler comme un château de cartes."

Another unique function and nomenclature were suggested by Vejdovsky (299, 139-147), who considered the first products of the mitochondrial granules to be the vacuoles and the remainder of the mitochondria to be used in the formation of reserve material as starch grains. The mitochondria first of all differentiate into "plastomerites" and "hyalomerites," the former being the formative substance, the latter being the rudiment of further differentiation. Thus the hyalomerites may develop into vacuoles or plastids, while the plastomerites by division into sister mitochondria again differentiate into plastomerite and hyalomerite, respectively.

Weier (310, 311) suggested that the "active" mitochondria described by Parat, Guilliermond and others were not mitochondria at all but artefacts produced by fixation, and that the plant plastidome was homologous with the animal Golgi apparatus.

Secretion

In 1905, Bouin (21) first suggested a secretory function for mitochondria in plants, but he identified the ergastoplasm of earlier writers with the chondriome. Guilliermond (105-108) gave momentum to the idea in 1913 with the results of his investigations on a number of fungi (*Penicillium*, *Botrytis*, *Endomyces*, various *autobasidiomycetes*, yeasts, etc.). In discussing their function he distinguished three kinds of reserve substances: glycogen, fat globules, and the "metachromatic corpuscles" of earlier writers. He claimed that the last arose from mitochondria as plastids do in the higher plants, every transition between mitochondria and metachromatic bodies being seen. Hence he ascribed to mitochondria the same secretory function as that claimed for these bodies in both plant and animal gland cells.

Using the mycelial hyphae, conidia and sexual organs of *Albugo blittii* and *A. candida*, Lewitsky (182) in the same year confirmed Guilliermond's observations. Meves (208) corroborated the observations of Guilliermond and Lewitsky and described the transformation of mitochondria into secretion globules in the aerial roots of *Chlorophytum*. Prenant (248) and Policard (241) both considered them producers and carriers of pigment in animal cells; the latter also observed them secreting fat, glycogen and pigments in plants, and was of the opinion that they were polyvalent in function. Milovidov (215) observed the stages from mitochondrion to starch grain, and Mangenot and Emberger (193) homologized them on the basis of physiological function in plants and animals; in the former they elaborate starch, in the latter haemoglobin.

Politis (243-245) ascribed the secretion of tannins, anthocyan and glucosides to the activity of mitochondria. In glucoside production, he described the mitochondria as swelling slightly at each extremity. This swelling increases, the mitochondria rupturing and separating and being transformed into spheres which augment their dimensions gradually, finally entering the vacuole where they exist for some time before being dissolved in the vacuolar sac.

Anthocyan is similarly formed and has a similar fate, according to Politis and Mirande (218).

Noel (231) and Tarwidowa (291) described droplets of fat arising from the chondriome and not from the cytoplasm as Guilliermond reported. The smaller fat globules, at first separate, later fused, completely surrounding the elaborating element. In permanent preparations, Tarwidowa described them as showing a net-like structure which is reminiscent of the trophoplast of A. Meyer. She denied the duality of the chondriome, maintaining that each mitochondrial element of the plant cell has the same potential function.

A further transformation of mitochondria into other granular inclusions of a different nature was described by Maximov (198). In fibrioplasts he observed the transformation of albuminoid grains into fat droplets, both of which arose from mitochondria.

Dangeard (61) in a further consideration largely of Guilliermond's work, said that he had shown that anthocyan and tannin were not elaborated by the mitochondria and chondriokonts but were the products of the vacuolar system and appeared in the interior of the spherical or filamentous primordial vacuoles of which Dangeard claimed to have first demonstrated the true nature. He concluded, perhaps too sanguinely, that "*Ces résultats mettaient fin d'une façon heureuse aux nombreuses discussions sur l'origine de l'anthocyane. . .*" Guilliermond (126) wrote:

The important researches conducted by P. A. Dangeard opened the way to a better understanding of the nature and evolution of vacuoles. These researches were suggested by our observations of the manner in which anthocyanin is formed at the margin of young living rose leaves.

E. V. Cowdry (43), investigating this problem, characterized as weak the doctrine of chemical transformation of mitochondria into substances of diverse constitution, put forward by Guilliermond and others. That there is ample evidence that mitochondria are active and fundamental in cell activity is conceded, but their actual part in the economy of the cell is obscure. They may have an entirely passive role in histogenesis and act only as a vehicle or substratum in which, by virtue of its physical and chemical properties, substances are deposited which are synthesized through the activity of the cell as a whole.

In a comparison of the mitochondria in plant and animal cells,

N. H. Cowdry (48-50) found them wherever protoplasm was active, save in few exceptions. Their progressive diminution and final absence in the later stages of the life cycle were also comparable. This diminution of mitochondria in plants was associated with the production of chlorophyll; in animals with the formation of haemoglobin—two substances of similar constitution.

A secretory function of the mitochondria in vessel formation was postulated by Alvarado (3) in 1919. He noted that:

1. With the formation of the large central vacuole and the location of the protoplasm along the wall of the cell, the protoplasm becomes more granular at the points where thickenings are to occur; and

2. The mitochondria develop considerably in exactly those large vascular cells where thickenings do occur; and

3. The secondary membrane is a secretion product of the protoplasm.

These three phenomena being simultaneous in the same cell, he suggested that the thickenings are due to the activity of mitochondria which are abundant in the cell immediately before the thickenings form. Alvarado's observations were never corroborated. In several fragmentary papers and with little apparent evidence, Causey (35-37) reported that mitochondria in *Euglena* arise *de novo* and disappear after completion of their function, which is secretory. The mitochondria in *Euglena* are spherical in form (37), whereas in *Noctiluca* they are both rod-shaped and spherical (36), the former occurring in regions of anabolic activity and the latter in katabolic areas. In *Euglena* they give rise to the pyrenoids, but in the parasitic flagellates to the parabasal bodies, according to Causey.

Respiration

The suggestion that mitochondria function in protoplasmic respiration was first made by Kingsbury (172) in 1912. He said that osmic acid, bichromates and formalin are the chief ingredients of mitochondrial fixatives and their chief value depends upon the presence of reducing substances in the cytoplasm. These he believed to be mitochondria on account of their lipoidal properties. Mayer, Rathery and Schaeffer (199, 200) concurred with this theory.

R. L. Lewis and W. H. Lewis (178) also suggested a respiratory function, and N. H. Cowdry (48, 50) concluded that they must be

regarded as an integral, perhaps essential, constituent of living matter. Their physiology is obscure, but their general similarity in all places may mean, in addition to specific functions like the production of chlorophyll, that they have a common part to play in some vital manifestation like protoplasmic respiration.

Their common identity in plants and animals may indicate a common ancestral form possessing mitochondria, and if their function is, as may be, respiration, he asks whether the presence of mitochondria in conjunction with a nucleus may not have made evolution possible, since there seems every reason to believe that the possession of formed mitochondria is an attribute even more primitive than the possession of a fully formed nucleus.

Prenant (249) reported an exclusive cytoplasmic distribution of peroxidase and its location in granules closely similar to mitochondria.

In a series of experiments between 1927 and 1938, in which new technics were used, Joyet-Lavergne (151-169) brought additional support to the theory of the respiratory function of mitochondria. He used reagents in which color changes were brought about by oxidation, such as leuco derivatives of cresyl blue, methylene blue, Nile blue, methyl blue, Janus green, and chemicals such as pyrogalllic acid, metol, cobaltous salts, hydroquinone, diamidophenol, *etc.* To demonstrate reduction, he used gold chloride, aqueous picric acid, potassium permanganate, dinitrobenzol, silver chloride and osmium tetroxide. The advantage claimed for these technics over the usual staining method which merely indicates affinity between chondriome and dye and is not specific, is that staining here is evidence of a physiological condition and is a revelation of the presence of an oxydo-reduction catalyst. The outline of the image is visible for only a short time, but in all cases the result was the same, *viz.*, "le pouvoir oxydo-réducteur du chondriome est une qualité très générale de ce constituant cytoplasmique." The nucleolus shared with the chondriome the ability to color leucobases. He concluded his researches with a statement claiming not only to have demonstrated that mitochondria play a role in respiration but to have illustrated the mechanism by which it is done. Joyet-Lavergne (167) also claimed to have demonstrated that mitochondria contain glutathione, probably performing the role of a catalyst of oxidation-reduction reactions.

Bourne (22) confirmed Joyet-Lavergne's findings and reported

granules and rodlets appearing in glutathione and vitamin A preparations which coincided with mitochondrial preparations. He suggested that mitochondria may be divided into two groups: those containing vitamin C and those not containing vitamin C. The former he believed to be ideally composed, chemically, to function as respiratory centers of the cell. According to Bourne, mitochondria are composed of an outer lipoidal cortex, which contains vitamin A and/or carotinoid pigments and a core (water-rich phase) in which vitamin C and glutathione are situated, these substances forming an oxidation-reduction system which is the basis of the activity of the mitochondria as respiratory centers.

Browne (33) has recently reported that the mitochondria of *Spirostomum* contain no vitamin C.

Enzymatic function

Working with proteolytic enzymes and dyes of the azine series, Marston (195) produced a new and ingenious theory, which has gained considerable support, as to the function of mitochondria. He observed that dyes of this series produced sparingly soluble compounds with a number of the proteolytic enzymes, and ascribed the precipitate to the action of the basic nitrogen of the azine nucleus. The linkage occurs through the basic nitrogen of the heterocyclic azine ring; this union institutes a tautomeric rearrangement within the azonium base, causing a color change. Janus green (diethylsafraninazodimethylaniline) has long been known, according to Marston, as a specific, only slightly toxic, stain for mitochondria, and its specificity is supposedly due to the proteolytic enzymes concentrated in these bodies. The mitochondria may thus be the site of synthesis in the cell, the water-poor phases which exist at the surface of the lipid constituents of the mitochondria instituting favorable conditions for the synthetic activities of the enzyme. This theory of enzymatic function of mitochondria is accepted by Piney (239) and Seifriz (275) as the most probable. ✓

Concurring with Marston, Robertson (257, 258) advanced the theory that mitochondria are loci of syntheses of the amino acids to proteins. Assuming the lipid-protein constituents of mitochondria, he pointed out that the lipoids would tend to orient the molecules of amino acids at the surfaces and bring the reactive amino and carboxyl groups into close proximity and to point them toward the aqueous phase; whereas the lyotropic hydrocarbon chains would

be buried in the lipid phase. The amino acids, except the heterocyclic ones such as histidine, proline, tryptophane and a few others, consist of the active groups $-COOH$ and $-NH_2$ attached to hydrocarbon chains of varying lengths. The effect of thus concentrating and bringing into close proximity the reactive groups would favor synthesis, according to Robertson, even if the catalyst were not in high concentration at the synthetic site. In a later paper, Robertson (258) suggested that mitochondria do not serve as a storehouse of amino acids but rather as a means of converting them when necessary into protein, the excess being stored in the nucleus.

Further support for the enzymatic theory of mitochondrial function was given by MacDougal and Moravsek (189) from their studies on colloids and protoplasm. They attributed the hydrolysis of the proteins in the young cell to the basic substances emanating from the mitochondria which begin to move from their position near the nucleus as the protoplast enlarges.

In a series of studies on mitochondria in various protozoa, Horning (140-146) made a number of interesting and perhaps significant observations. He distinguished mitochondria from bacteria by the use of a new stain (a sodium salt of diethyl safranin monocarboxylic acid, prepared from Janus green by the hydrolysis of the nitrile) and demonstrated that mitochondria cannot be symbiotic organisms. The number of mitochondria in a single cell varied considerably. They were observed to group around and within the engulfed food particles and this was regarded by Horning as a direct demonstration of the origin of digestive enzymes from mitochondria. Browne (33) also observed the presence of mitochondrion-like bodies around the food vacuole of *Spirostomum* but interpreted them as bacteria.

Later, Horning and Petrie (147), in their studies of the function of mitochondria in the germination of certain cereals, offered further evidence in support of their earlier work on protozoa and that of Marston regarding the enzymatic function of mitochondria. Wherever they found indication of enzymatic activity in the solution of starch in germinating cereals, the enzyme appeared to be localized in the mitochondria, and when the enzyme was secreted it was translocated likewise in the mitochondria and was liberated therefrom only when it reached its substrate. They also observed a comparable relation between the numbers of mitochondria and extent of enzyme action. This apparent affinity of mitochondria

for starch grains just prior to and during hydrolysis is interesting, and the theory proposed is suggestive. Corroborative evidence with minor variations was reported by Newcomer (226) in germinating seeds of *Zea mays*.

Symbiointicism

In 1905, Mereschowsky (201) published a paper in which he concluded that the chromatophores of plants, like the green cells found in various animals, were symbiotic organisms representing the blue-green or green algae which originally entered the cell as endophytes and which have become symbionts or helots, as is the case of the lichen thallus.

A similar view of mitochondria was later presented by Portier (247), who maintained that mitochondria were really bacteria and that he had isolated and cultured them. His technic and results met with little acceptance among his contemporaries, and Wallin (307, 24) was of the conviction that Portier's "organisms" exhibited properties incompatible with the known properties of living matter or protoplasm.

Further support to the concept of mitochondria as symbionts was advanced by Wallin (307) after a series of very extensive and careful investigations between 1919 and 1927, from which he concluded that mitochondria were bacteria living in the cytoplasm of all higher organisms and that their symbiotic existence had its inception at the dawn of phylogenetic evolution. He compared mitochondria with bacteria in their staining and chemical reactions, reactions to physical agents, thermal responses and chemical constitution, and reported them similar if not identical. He further claimed to have succeeded in growing mitochondria in culture media. He said:

These facts, apparently admit of no other interpretation than that mitochondria are living organisms, symbiotically combined with the cells of plants and animals . . .

Microsymbiosis appears to be a universal biological phenomenon. The cells of all normal plants and animals contain minute bodies which have been named mitochondria. In a series of researches the author has been able to demonstrate the bacterial nature of these bodies. Their universal presence in the cell, coupled with the well known properties of bacteria, appear to indicate that mitochondria represent the end adjustment of a fundamental biological process. The establishment of intimate microsymbiotic complexes has been designated "symbiointicism" by the author. Symbiointi-

cism, then, is proposed as the fundamental factor or cardinal principle involved in the origin of species.

Keller and Oparin (233), from purely theoretical considerations, considered the cell as a symbiotic complex. The former wrote:

This symbiosis of organisms, which was at first accidental, gradually became elaborated into a most intimate and permanent system in which the previously independent organisms acquired the character of organs of a single whole, the cell.

And Oparin (*loc. cit.*, p. 198) said:

. . . it seems much more probable that the origin of the isolated nucleus, chondriosomes, plastid, *etc.*, is only the external visible expression of a gradual unfolding and perfection of an inner physico-chemical structure and organization of colloidal formations.

Professor East (70) was of the opinion that:

. . . the theory of Portier and Wallin should be given serious consideration, since it is susceptible of proof through culture *in vitro*. The criticisms offered against it by members of the micro-dissection school, while grave, are not wholly destructive.

The distinction between mitochondria in plant and animal cells and contemporary species of bacteria was rather convincingly demonstrated by E. V. Cowdry (44) and Cowdry and Olitsky (47). The mitochondria of living lymphocytes and five different types of bacteria were compared in their reaction to vital staining. They reported mitochondria staining in a solution of Janus green B as dilute as 1: 500,000, whereas the bacteria could not be stained in a solution more dilute than 1: 60,000. Comparisons were also made of their respective reactions to various killing fluids and staining with haematoxylin, acid fuchsin, and Giemsa's stain in the tissues of rabbit pancreas into which had been injected four kinds of bacteria. In general, the bacteria survived fixations which contained acetic acid or alcohol in appreciable concentrations, whereas the mitochondria did not. Staining differences were also reported, especially with Giemsa's stain, and further distinctions in morphology were observed.

Plasmon

Wettstein has applied the term "plasmon" to the genetical elements of the plasm as contrasted with the term "genom" by which Winkler had denoted the whole collection of genes contained in the

chromosomes (283). The first suggestion that mitochondria may function in heredity was made by Meves (203) in 1904. He considered mitochondria as forming the material for the various processes of differentiation which the tissues undergo in development from the fertilized egg. Meves did not consider these bodies as *the* inheritance carriers, to the exclusion of the nucleus, but suggested that the nuclear qualities were conveyed by the chromosomes and the cytoplasmic qualities by the mitochondria. Voïnov (300) concurred, and Mottier (221) also suggested that the nucleus may not have a monopoly in heredity. Of the many transmissible characteristics which cannot as yet be definitely expressed in any Mendelian ratio, he suggested the explanation of their being carried by these less conspicuous cell organs. In support of his theory he asked whether, if as some have attempted to show, the pigments grouped under the term "anthocyanin" are caused by a definite granular or rod-shaped body which is a permanent organ of the cell, we are to conclude that colors—whether behaving in the Mendelian ratio or not—are transmitted by the nucleus. Dangeard (50) also was of the opinion that the presence of spherome and plastidome in pollen and embryo sac must be considered in the genetic transmission of hereditary characters.

On the other hand, Hogben (138) said that Mottier's ideas on mitochondria functioning in heredity cannot be treated with serious consideration, and E. V. Cowdry (43) opposed the concept of mitochondria as functioning in heredity, regarded as probable by Jenkinson, Conklin and Wilson. It would be hard to conceive of phospholipins as carriers of heredity, even though they contain albumin. Chemically, chromatin appears best fitted to play the part of carrier, concluded Cowdry. Goldschmidt (91) and East (70) also believed that:

... it is ordinarily unnecessary to visualize the cytoplasm as a vehicle for carrying hereditary factors. The cytoplasm behaves as a neutral agent.

Other functions

Several other functions have been ascribed to mitochondria, none of which has received wide acceptance. Among the earlier workers, Arnoldi and Börnicke (7), Scherrer (269) and Devisé (64) considered them as serving a nutritive function, and Dangeard (55) suggested a basic role in cell metabolism. They were considered

by Regaud as centers of specific chemical action, like the plastids, which serve to extract, elaborate, and fix definite chemical constituents of the protoplasm. According to this eclectosome hypothesis, mitochondria are plasts choosing and selecting substances from the surrounding cytoplasm, condensing and transforming them in their interior into infinitely diverse products. Mitochondria are almost, although not quite, coextensive with vital phenomena. In the cells of all embryos they take part in vital activities of a generalized and fundamental type before the onset of specialization.

Nicolosi-Roncati (228) ascribed to them the role of equal distribution of chromidial substance between the two daughter cells in the pollen mother cells of *Helleborus*.

GENERAL CRITIQUE

The late Professor East (70) recently remarked, with his usual accuracy and succinctness, that

Inquiries into the precise significance of mitochondria have fallen into disfavor with all but a hardy few, and not without some justification. The topic has been too prolific of unsupported generalizations. The single topic fallen lower is that of the Golgi apparatus and the nadir is reached when the two are homologized.

Another critic of unsupported generalizations said that the naive attitude on the part of some investigators, who seemed to feel that the value of their own interpretations of observations was increased by frequent repetition and that they had reached the limits of cytological technic, reminded him of the Bellman in "The Hunting of the Snark":

Just the place for a Snark. I have said it thrice: What I tell you three times is true.

The literature is replete with polemic dissertations based perhaps too frequently upon analogy and *a priori* considerations. The reluctance of the investigators to agree upon a generally acceptable nomenclature, for which Bowen pled unsuccessfully, is a serious obstacle to the successful solution of the problem. The present status seems to be the existence of four major systems of terminology, with minor modifications, and the contributions from these four schools consist largely of support and reiteration of views previously expressed. This narrow adherence to a doctrinaire position and, to the uninitiated, this insistence upon apparently

metaphysical distinctions between categories of cytoplasmic constituents which are morphologically and chemically similar, have contributed to make the subject esoteric.

The problem at present stands essentially where Lewitsky left it in 1910, when he adequately demonstrated by photographs of fixed and fresh material, the transformation of some of the chondriome into plastids. Other suggested functions of mitochondria, although suggestive and attractive, are in need of corroborative evidence.

This partial transformation of the chondriome into plastids raises the obvious question whether, as the Schimper-Meyer group maintains, there has not been a confusion of juvenile forms or pro-plastids with mitochondria and whether they may not be separate entities. In view of the variability in kind and number of plastids in the various cells, the many excellent photographic illustrations of plast formation from mitochondrion-like bodies, and the further fact that all evidence thus far produced for the separation of the chondriome and plastidome in meristematic cells seems unconvincing and inconsistent, it would seem unwise to separate the chondriome into "active" and "inactive" constituents, pseudochondriome-plastidome, cytome-plastidome, *etc.*, no matter how desirable from *a priori* or homology considerations. The polymorphism of mitochondria in meristematic tissues, the dangers inherent in preparing such fragile structures for observation *in vitro* as well as the conditions of observation, render futile any attempt to devise a system of individual nomenclature based upon morphology to embrace all forms.

Bowen (28) attempted to separate the chondriome into pseudochondriome and plastidome on the bases of morphology, their orientation during cell division, and staining behavior, and concluded that polymorphism was a property of the plastidome. Duplications (226) of Bowen's experiments on the same and other materials have definitely shown the inadequacy of any such separation by these methods. Leucoplasts frequently arise from a granular chondriome, filamentous mitochondria are present in the root-cap cells, there were no consistent distinctions in staining behavior, nor was there any peculiar orientation of the plastidome during anaphase of mitosis. The latter may frequently be caused by the polar orientation of the vacuoles at anaphase, resulting in radial arrangement of the cytoplasmic strands separating the vacuoles, so that mito-

chondria situated in these areas are radially arranged not by virtue of membership in the plastidome but of necessity.

The occasional survival of portions of the chondriome after fixation with strong Flemming's or the equivalent would merely seem to indicate that certain members of the chondriome vary in protein content or that these survivals are actually young plastid stromata. The variability in the image of the chondriome produced by such fixation renders it useless as a criterion of separation. The Kolatchev technic and modifications of osmic impregnation methods likewise yield variable results and are unreliable. The variability in the degree of unsaturation of the lipins present in mitochondria in the same and different tissues must seriously diminish the reliability of such technics, for osmic acid will blacken any given mitochondrion or portion of it in direct proportion to its degree of unsaturation (190). Cytological technics are necessary and important, but their capriciousness requires caution in interpretation. Walker and Allen (306) are of the opinion that:

The Golgi elements and apparatus, and some of the structures described as chondriosomes in preparations treated with osmic acid, are probably artefacts and produced by the methods used to demonstrate them.

The osmiophilic platelets can also be placed in this category for they seem to be but the blackened remnants of a chondriome deformed by the initial fixation. If Zirkle's fluid be substituted for Champy's in the initial stages of the Kolatchev technic, the chondriome is beautifully preserved. Indeed, the technic is almost specific for the chondriome, bleaching alone being necessary after sectioning.

The insistence upon the duality of the chondriome has been based largely upon two *a priori* considerations. The first is that if, as there seems good evidence for believing, the chondriome of animal cells is homologous with that of plant cells, it is difficult to homologize the function of plast formation with any function of mitochondria in animal cells. N. H. Cowdry (48) suggested the secretion of haemoglobin for this desideratum, but the theory has not been generally accepted. The idea seems worthy of further consideration and seems preferable to the often suggested homology of the plant plastidome with the animal Golgi apparatus, about the latter of which virtually nothing is known and whose real existence is doubted by many competent investigators. Gortner (93) cites

some suggestive evidence which supports Cowdry's theory. The former stated:

If one compares the formula of aetiophyllin with the formulae which have been suggested for hemin, it will be observed that there is a very striking similarity in the structure of these two pigments, the one the vital pigment of the autotrophic plants, the other the vital pigment of most representatives of the animal kingdom. It is highly improbable that the close similarity in the structure of these two vital pigments is one of chance, but it appears more probable that in the processes involved in organic evolution, the essential nucleus of the earlier vital pigment, chlorophyll, became modified so as to assume new functions in the developing animal kingdom, the Mg in the chlorophyll being replaced with Fe (or Cu in the hemocyanin of the Crustaceae) in order to care for the new function, as an oxygen carrier, the branched-chain aliphatic alcohol, phytol, being similarly replaced by a protein residue (a histone, globin) possibly because the animal body cannot synthesize such compounds as phytol but can reconstruct a protein molecule from the amino acids which are secured from the food.

The above hypothesis is strengthened by the observation of Küster, who suggests that haemoglobin is in reality a mixture of two compounds, haemoglobin *a* and haemoglobin *b*, differing only in the fact that one has a free carboxyl group, whereas in the other this carboxyl group is internally linked within the molecule, thus affording an even closer parallelism to the chemical structure of haemoglobin and chlorophyll.

Both chlorophyll and hemin yield "porphyrins" when acted upon by acids. These porphyrins are Mg and Fe free, respectively. Chlorophyll *a* and chlorophyll *b* yield the same porphyrins, *and these are isomeric with those formed from hemin.*

The second *a priori* consideration suggesting the desirability of a dual chondriome is that in the Bryophytes and Pteridophytes, plastids and mitochondria have been reported to exist side by side throughout the life cycle, neither losing its individuality. Between the Pteridophytes and Spermatophytes, however, there is apparently a sharp break in the phylogenetic series in the development of plastids. In the Spermatophytes they originate from plastid primordia which appear mitochondrial in nature. Since the mature, photosynthetic tissues of Spermatophytes carry, besides chloroplasts, bodies similar in morphology and chemical constitution to those of the lower plants as well as animals, it would appear that phylogenetic alteration had occurred only in the transmission of the plastidome and that these mitochondrial bodies of the mature cell

constitute the plant "pseudochondriome" or "inactive" chondriome or "cytome" which is homologous with the chondriome of the lower plant and animal cells.

Objections to this point of view lie in the facts that the plastidome and chondriome do not always maintain their individuality throughout the life cycle of the Bryophytes and Pteridophytes and Emburger and others have reported plastid reversion to mitochondrial form in the lower plants as well as in the Spermatophytes.

The more recent evidence seems to support the earlier hypothesis of Regaud, namely, that mitochondria are in a large measure eclectic in nature—not autonomously, perhaps, as suggested by Regaud, but functionally versatile subject to the general requirements of the cell or tissue. The studies of Faull (77) and Newcomer (226) have shown the development of mitochondria into elaioplasts, leucoplasts, chromoplasts and chloroplasts in different tissues; the observations of Horning and Petrie (147), Robertson (257), Marston (195), and the writer have indicated a possible relationship between mitochondria and cellular enzymatic activities. These observations, as well as the fact that over eighty products of secretion have been ascribed to mitochondria in plant and animal cells, all seem to subscribe to the functional versatility of mitochondria and to the belief that the arbitrary separation of the chondriome in plants into two categories is no longer adequate.

The objection that there is no body in the animal cell comparable in morphology with the chloroplast in the plant cell does not seem a valid one in view of the well known pleomorphism of mitochondria in both plant and animal tissues, their variability in development and the differences in the general metabolism of the two groups of organisms. The apparent identity of plant and animal mitochondria in chemical composition and morphology; their observed morphological transformations into bodies of different size and structure performing varied functions in the plant and animal body; the reversibility of chloroplasts to mitochondria, *etc.*, all seem to point to the concept that the chondriome of animal cells and the plastidome, pseudochondriome, active and inactive chondriome, *etc.*, of plant cells, are but developmental stages of a single category of cell organ, namely, mitochondria. As such, the chondriomes of plant and animal tissues are homologous. The argument for the homology of the plant and animal chondriomes and their essential, fundamental identity, modified as expected by the

vicissitudes of their respective phylogenetic development, seems to rest on the firm foundation of functional similarity or versatility as well as chemical and morphological identity.

No answer can be given to the question why mitochondria existing side by side in the same cell differ so greatly in morphology, unless the answer is found in their peculiar property of responding to the various metabolic requirements or activities of the protoplast.

In plant cells no real chemical distinction has been made between any types of mitochondria, and they apparently consist of identical materials. That there is a chemical change concomitant with the development of mitochondria into plastids is indicated by the greater tolerance of the latter to fat solvents such as acetic acid and alcohol. Apparently the resistance is proportional to their degree of differentiation, mature plastids being very tolerant to fat solvents.

Physically, mitochondria are apparently a highly hydrated mass of lipo-proteins with a specific gravity and viscosity slightly greater than that of the surrounding cytoplasm but frequently exhibiting a semi-fluid condition in areas of high cellular metabolism. The fact cannot be without significance that they possess a high surface area and consequent high surface energy and thus provide the cell, by this structural differentiation, an ideal arrangement for the performance of a variety of physiological or metabolic activities proceeding side by side without interference with one another.

Since lipoids decrease surface tension and mitochondria are largely lipoidal in structure, their frequently observed position surrounding the nucleus, plastids, starch grains, or lying closely appressed to the cell walls may be thus accounted for. But their migration from place to place in the cell also suggests a status more dynamic and significant than that of a purely ergastic body.

The following alternative hypotheses, both of which are to some degree consonant with experimental evidence, are here presented as a tentative basis for the consideration of the fundamental nature of mitochondria.

1. The autocatalytic property of mitochondria may seem apparent from their reproduction by division and the absence of evidence of *de novo* origin. If, then, they possess the heterocatalytic properties ascribed to them by Robertson, Horning and Petrie and others, we have a self-perpetuating and self-duplicating particle which, according to Alexander and Bridges (1), we must consider as living.

This conception of mitochondria as bionts is further supported by their sensitivity to hydrogen-ion concentration, temperature and traumatic effects. Gatenby (85) flatly stated that mitochondria are able to assimilate, grow and divide in the cytoplasm as a protist does, and he ascribed to them a marked degree of independence but held that they are not symbiotic. This is essentially the view originated by Altmann and elaborated by Wallin, although the latter carried it to its logical conclusion and ascribed to them a bacterial nature and a symbiotic relationship to the cell.

2. On the other hand, ascribing to mitochondria such a degree of autonomy and individuality as cell organs *sui generis* may be an exaggeration of their significance. That they may constitute merely loci instead of agents of reactions, and that their true function may be only an expression of the metabolism of the adjacent cell organs acting upon them, is not without semblance of validity. Instead of the assumption that they are endowed with life properties and as such are the ultimate constituents of the cell, may be advanced the concept that they are lifeless structures, perpetuated, it is true, throughout the life cycle of the organism, but passively, and that their apparent growth is the result of physical accretion or adsorption and their division merely the working of physical forces activated by the relationships between surface tension, volume and surface area. In other words, their apparent attribute of life is not a property peculiar to mitochondria but is the result of association and interaction with other components of the cell, which, though individually lifeless, constitute a living substance when combined. From this point of view they must necessarily be considered as merely loci of reactions and not causal agents. As such they furnish the cell with mobile, discrete units high in surface area and energy, thus constituting ideal surfaces for reactions, the adsorption of enzymes, and the transportation of the enzymes to the various substrates, permitting and facilitating the complicated reactions of cellular metabolism. They thus differ from ergastic bodies in being not the result but the *sine qua non* of metabolic activity.

As to their ultimate origin, they may very well be cytoplasmic condensations resulting perhaps from physico-chemical forces in the cytoplasm. This concept of mitochondria is not violated by their chemical nature as the cytoplasm is also largely a lipoproteid complex in an aqueous salt solution, the distinction being solely one of particle size or phase of solution. Their greatest prevalence in

meristematic tissues which are usually high in both metabolic activity and viscosity may not be without significance.

Furthermore, this theory is not invalidated by the well known fragility of mitochondria and their extreme sensitivity to changes in osmotic pressure, pH, traumatic effects, *etc.*, if one takes into consideration the fact that protoplasm is an emulsion probably existing very near the breaking point, where slight chemical or traumatic stimuli could easily cause phase reversal or any of the phenomena attributable to the fragility of mitochondria. As such, the fragility of mitochondria may be similar to that observed in thixotropic gels or artificially produced emulsions.

Distinctions between what we may loosely call these "vital" and "non-vital" concepts of mitochondria seem to be supported by experimental evidence, which in the author's opinion favors the latter theory. Any consideration of mitochondria as bionts or cell organs *sui generis* seems to necessitate a sharp distinction between the plastidome and pseudochondriome confining the property of polymorphism to the plastidome, as it is obviously incredible that the same cell organ should exhibit such variations in development. If this distinction is made, we have two categories of cell organs sharply defined in function but indistinguishable in chemical nature, structure and morphology at certain stages. This is *prima facie* improbable and is unjustified, in my opinion, by the best evidence.

That mitochondria are not living cell organs but lifeless constituents of the cytoplasm seems to be suggested by their chemical nature and identity of all types, their pleomorphism in different tissues, their abundance in meristematic cells and decrease in numbers in mature cells, which is not adequately explained by plastid formation at their expense. This concept seems further supported by their chemical change concomitant with functional differentiation, *i.e.*, from mitochondria to plastids, *etc.*, which is hardly conceivable of living cell organs and has no parallel in the ontogeny of micro-organisms. Their absence in many mature cells, as has been frequently reported, and their great abundance in young tissues of high metabolism suggest a correlation with cellular activity more evanescent in nature than cell organs *sui generis*.

SUMMARY

It is plain from this survey that the opinions of the most competent investigators are in conflict on many important points. The

tendency of many workers to dogmatize upon the physiological implications of the chondriome from morphological studies alone and to accept too implicitly the image presented by a cell fixed with various chemicals without adequately studying the effects of these chemicals upon the polyphase, colloidal system comprising the protoplast, has not contributed to a clearer understanding of the problem. There has been a plethora of isolated investigations and interpretations but no new experimental technic has brought us noticeably nearer to a solution of the problem.

The significance of mitochondria in metabolism, reproduction and evolution, and their status in the cell community, whether *sui generis* or evanescent in nature, remain to be more adequately demonstrated. Opinion is still divided concerning them, and there is an equivalence of testimony thus far for many theories.

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AERATION AND PLANT GROWTH IN WET SOILS

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Botanists have long accepted the idea that the roots and other submerged parts of aquatic and marsh plants are exposed to much lower oxygen concentrations in the surrounding medium than the corresponding parts of land plants. It has been assumed that the presence of aerenchyma in the plant tissues allows oxygen to diffuse down to the submerged organs from the atmosphere through the aerial parts of the plant. This assumption is plausible enough as a qualitative statement, but ecology has the task of verifying it experimentally and making it as far as possible quantitative. The effort to do this has revealed, and may still reveal much more closely, the subtle relationships between plant and environment in these "amphibious" habitats.

The question has not often been attacked as a whole, and the data seem at first sight rather diffuse. On the one hand, there are researches which deal with measurements of oxygen concentrations in soil and water under natural conditions, and, on the other hand, there are investigations of the degree of tolerance of different plant species to various artificial soil conditions applied experimentally. These two aspects blend together to support the earlier qualitative picture, but the most recent research that bears on this subject, that of Pearsall and his co-workers, not only involves this picture, but goes beyond it in opening up fresh complexities in the ecology of marsh and water plants.

It is not difficult to summarize briefly our knowledge of actual oxygen concentrations which are present in different soil types, because of the extensive survey of all the work previous to 1922 which has been made by Romell (13). The theoretical part of his paper deals at length with the problem of gaseous exchange between the air spaces of the soil and the atmosphere above the soil, and Romell concludes that the oxygen concentration in the soil spaces is much more effectively maintained by the process of dif-

fusion than by factors such as temperature and pressure changes which would tend to bring about mass movement of gases. On the experimental side Romell gives a large number of values of oxygen and carbon dioxide percentages derived from analyses of samples of the soil atmosphere. He covers the work of many investigators over a wide range of soil type and geographical region, and, in addition, his own data give much information concerning Swedish soils.

Some of the results deal with marshy soils with a fairly high water table, and analyses are given of gas samples taken from a point just at the level of the water table, as indicated by the presence of water along with the gas sample. In these cases the oxygen concentrations nearly always fall below 15%, and are often very low indeed, values of less than 1% being recorded. Higher levels in the same soils gave samples with the usual high values of 15% or over, and it is clear that there is a very sudden change in conditions of oxygen concentrations within a narrow region of soil, which delimits the zone of complete saturation. The carbon dioxide values in this limiting region are correspondingly high, and one has to postulate either a very high rate of decomposition of organic materials, where these are abundant, or a very great resistance to gaseous exchange.

Exactly the same sharp change is apparent in the data of Russell and Appleyard (14), whose analyses of gas samples from cultivated soils always give oxygen values over 18%, except when the sample is obtained from just above a water table. The dissolved oxygen content of the upper layers of the water table is most unlikely to have a value higher than that which would be in equilibrium with the gaseous concentration immediately above it. Estimations of dissolved oxygen in soil water do not seem to be very numerous, but a number were carried out by the writer for the peat soil of Wicken Fen (East Anglia), and this idea was fully confirmed, since the concentrations never exceeded one-tenth of the value for equilibrium with the ordinary atmosphere.

This condition probably holds good as a general rule wherever there is a water-logged soil, and hence one may conclude that roots and rhizomes which are actually situated in such soils are undoubtedly subjected to a medium with very low amounts of free oxygen. It seems more doubtful, however, that the same conclusion applies to those parts of the plant which are submerged in water but not

necessarily buried in soil or mud, for instance, the stems and submerged leaves of *Nymphaea* and other true aquatics. The water round the plants, at any rate the upper layer, often contains considerable quantities of dissolved oxygen, particularly where active photosynthesis may take place. Bergmann (2) gives some data which are interesting in this connection. Samples of water from a depth of 2 feet in Lake Hubert, Minnesota, had dissolved oxygen contents round about 6 to 7 cc. per litre; temperatures are not given, but these values were probably close to those for equilibrium with air. For samples taken from the surface waters of swamp lakes, he found lower values (3 to 4 cc. per litre), but even these are much higher than any results for samples obtained from below the soil surface, as far as can be judged from the literature which has been examined by the writer.

The measurement of oxygen concentrations, whether in the soil atmosphere or in solution, is not the only criterion of soil aeration, and though it is the most direct way of investigating the supply of oxygen to aquatics it is important to keep in mind the indirect effects of an oxygen deficit.

There are numerous studies of the way in which soil characteristics are affected by bad drainage or by artificial submergence, as, for example, the work of Robinson (12). He gives evidence that submerged soils contain abnormally high concentrations of manganese, and also of ferrous ions which render the soil solution toxic to most species. These substances are kept in solution as bicarbonates on account of the high concentrations of carbon dioxide which result from the submergence of soil, and the presence of Fe^{++} ions is a symptom of highly reducing conditions, demonstrated also by the frequent presence of sulfuretted hydrogen and sometimes even of free hydrogen. Robinson did not find that submergence affected the pH values very markedly: if anything, they fell slightly, perhaps owing to the higher CO_2 concentration, but in the water-logging experiments of Subrahmanyam (15) there was, on the contrary, an initial rise in the pH, accompanying a marked increase in ammonium content. This indicates the reducing conditions brought about by water-logging, and so also does the rapid absorption of oxygen by the soils which was observed by Subrahmanyam.

Gillespie (6) was one of the first to apply to soils the idea of measuring reduction intensity directly by electrical methods instead

of deducing it from the estimation of reduction products. He demonstrated the increase in reduction intensity which is brought about by submerging a soil and suggested that this reduction intensity itself is toxic to many plant species, apart from the chemical substances which are the result of it. He showed also that this method can give a wide range of readings in conditions where direct oxygen estimations would reveal very little, since the quantities of free oxygen are negligible.

Before going on to discuss the most recent work on this line we may consider the knowledge gained by experiments with plants. The work of Bergmann (2) can be taken to illustrate the type of result frequently obtained. He showed that hydrophytes, such as *Ranunculus abortivus* and *Cyperus alternifolius*, have better growth when grown in submerged soil, whereas land plants, as *Impatiens balsaminea*, showed decreased water absorption by the roots, with reduced vigour and finally death of the whole plant. If, however, the water round the roots was aerated, these ill-effects were prevented. Bergmann considers that the marsh species can tolerate the lack of oxygen because of the presence of aerenchyma in their roots, but does not, in the paper cited, bring forward any evidence for this view. He discusses the question of the limiting supply of oxygen necessary for the successful growth of different plant species, but his own experiments are not of such a nature that they can shed light on this point, partly because most of the conditions used were extreme, and partly because actual oxygen content was not determined quantitatively except as a check when good aeration was required.

Cannon (3), however, has provided extensive data which deal with the question of limiting oxygen supply for a number of species, covering plants of very varied life form and ecological requirements. He measured the growth in length of roots in moist soil under various oxygen concentrations and compared it with the growth under normal conditions where the soil atmosphere had a composition close to that of air. He distinguished a lower and an upper critical concentration; at and below the former value, all growth was inhibited; at and above the latter, growth was the same as in normal conditions.

The lower critical concentrations were in nearly every case lower than 7%, and for temperatures below 25° C, the upper critical con-

centration was usually below 10%. Cannon found that hydrophytes, such as *Salix* spp. and *Zea Mais*, had considerably lower critical concentrations than land plants, such as *Phaseolus*.

Zimmerman's experiments (17) on the root production of cuttings gave similar results, and a table given by Romell (13), summarising a number of other observations of this kind, points forcibly to the conclusion that, for the majority of plants, oxygen concentrations must fall to 10% or 11% in a soil atmosphere (or the corresponding concentration in solution) before any injurious effect is produced. Although, as already discussed, such low values are rare in ordinary soils, they are almost invariable in the submerged soils of aquatic habitats. The question has therefore arisen as to whether or not the roots of plants growing in these conditions are able to grow and develop with a smaller supply of free oxygen for respiration than is needed by plants of other habitats, or whether they do in fact need the same supply, but obtain it not from the immediate environment, but indirectly from the air by way of the internal aerenchyma of the plant.

Though this latter view has long been accepted, it is one that should rest on experimental verification, because it is conceivable that the presence of aerenchyma is not an adaptive character but a tendency in the development of normal parenchyma when it differentiates in a plant organ which is surrounded by water and not by air. That this character is genetically fixed in many species does not prove that it is essential for the supply of oxygen to the root system. We see, for example, an extreme development of aerenchyma in a freely floating aquatic, such as *Pistia Stratiotes*, but this plant lies right at the surface of open water, and it is unlikely that any part of the plant is growing in an oxygen-deficient environment.

For any given species the answer to this problem must come from direct investigation of the air-space system of the plant itself, to find out in the first place whether it is in fact a "system," that is, whether it is continuous through the plant body, and in the second place, what concentrations of oxygen are actually maintained in the atmosphere which occupies the air-spaces. References to the earliest results on this subject are given in a paper by Barthelmy (1).

He quotes some work by Dutrochet in 1837 which gave the following analyses of the gas from the internal air-spaces of *Nuphar lutea*:

	Percentages	
	Nitrogen	Oxygen
Rhizome	84	16
Root	82	8
Leaves	82	18

The oxygen percentage for the root is here fairly low, in contrast to the value of 14.1% which is quoted from the work of Martins and Moitessier for the roots of *Pontederia crassipes*. The same author gives the range of values for the roots of *Jussiaea* as 8 to 15%. Barthelmy himself gives various values for the leaves of *Nelumbium speciosum*, including one as low as 10%; he does not, however, deal with roots, and perhaps his most interesting contribution is a demonstration of the fact that in this species the air spaces are continuous with one another, at any rate in the stem and leaves.

Systematic investigations of the air-space system have lately been carried out on *Cladium Mariscus* (4), an inhabitant of reed swamps and wet fens. This species has evergreen linear leaves growing up into the air from a short rootstock which, when the plant is growing in its natural habitat, is situated below the soil surface and generally well below the water surface. The leaves are traversed by large internal air-spaces and the roots also are normally composed largely of aerenchyma, giving them a fleshy appearance. Only in the abnormal cases where the roots develop above the level of the water table are they fibrous and much branched.

Roots and leaves are both attached to the rootstock in which the tissues are very much more compact, but it has been shown that gas can pass under pressure from the leaves to the roots and also to the horizontal rhizomes by which this species is vegetatively propagated. This applies, however, only to the mature leaves, that is, those that are entirely differentiated and no longer growing.

There are, however, a number of young leaves immediately surrounding the shoot apex which are tall enough to expose their upper, differentiated regions to the atmosphere and so presumably to carry on assimilation, but which still have the active intercalary meristem by which they grow out from the base, according to the common monocotyledonous pattern. These leaves offer a greater resistance to the passage of gas into the rootstock and so to the

roots. It was therefore possible to plan experiments in which plants could have the main path of passage of air to the roots blocked up by cutting short the mature leaves and submerging the cut ends, while retaining at any rate part of the assimilating leaf area.

The general line of the experiments and their results is suggested by the examples in the following table:

Treatment of plant	Dissolved oxygen content in water surrounding roots and base of plant. Mean value in mgm./litre.	Percentage of oxygen in gas obtained from internal spaces of the roots. Mean of three plants in each case.
1. Plants entire; roots, root-stock and bases of leaves submerged in water to which air has free access	3.92	17.03
2. Plants as above, but water deficient in oxygen and covered with a thick layer of paraffin	0.48	17.06
3. Plants with all leaves, except the inner growing ones, cut short below the level of the water surface. Water as in 1	3.91	3.45
4. Plants as in 3. Water as in 2	0.08	1.21

The first two results show clearly that the roots of a normal plant do not depend for their oxygen supply on that in the medium surrounding them, and the effects of interfering with the air-space system are strikingly exhibited by the low oxygen values in the last two cases. These results are only examples of a much larger number which all lead to the conclusion that with the plant in its natural condition, oxygen can diffuse down to the roots from the sub-aerial parts, in this case the mature leaves, at a rate which is sufficient to maintain concentrations of 15% and over in the internal air-spaces of the roots.

This value lies well above the 10% or 11% which seems, from the data previously discussed, to mark the upper limit of oxygen concentrations which are likely to be low enough to injure the majority of plant roots. As far as *Cladium Mariscus* is concerned, therefore, there can no longer be much doubt as to the significance

of the internal air-space system in relation to the supply of oxygen to the roots. This species is not outstandingly rich in aerenchymatous tissue in comparison with the majority of plants growing in a similar habitat. In fact, when one examines the tough woody root-stock of the plant it seems surprising that gases should diffuse through it with the degree of ease which is demonstrated by the experimental results. It seems probable on the whole, therefore, that the conclusion reached about *Cladium* would hold good also for other helophytes showing the same degree of development of aerenchyma. Such species as *Carex riparia* and *Scirpus lacustris* show a similar type of habit, with a long-submerged growing point, and might be expected to yield results parallel to those for *Cladium*. *Phragmites communis*, though somewhat different in habit, would probably, like *Cladium*, show relatively high values of oxygen concentration in its roots, under natural conditions, since its almost pipe-like structure should offer an easy path for diffusion of gases.

The situation seems less clear when one comes to consider the root systems of tree species which typically inhabit wet soils; it would be very desirable to have data on the oxygen supply available for the roots of such species as *Alnus glutinosa* and *Salix fragilis*. These trees would normally belong to a somewhat later stage in a hydrosere than *Cladium Mariscus*, and may be subjected to somewhat different conditions of soil aeration, or may have different oxygen requirements.

It is to these border-line cases that the centre of interest is bound to shift if we accept the idea that the internal "aerating system" of the extreme helophytes makes them independent of the oxygen concentrations round their submerged parts. For we shall want to know what type of soil, or rather, what degree of aeration of a soil is just sufficient to allow the roots of the plants growing in it to be independent of an oxygen supply obtained from the aerial parts of the plant.

Between this point and the position of the *Cladium* type, there may be every grade of partial dependence on an internal atmosphere, and we should like to know whether in fact any species belong to this transitional type, and if so, which they are. Such species would belong to the intermediate stages of a hydrosere. One of the main features of a hydrosere must be the change in the relative levels of soil and water, and in the earlier stages this relationship

must differentiate the plant species of a succession according to their degree of tolerance to oxygen deficiency in the medium in which they are growing. In the later stages other factors must predominate, and the question is, where is the dividing line between earlier and later stages, in this sense?

Recently Pearsall and his colleagues have published data dealing with redox potentials which have a close bearing on the problem just expressed, though they do not give a definite answer to it, partly because they cover a much wider field of investigation (8, 10, 11).

The theoretical basis of the redox potential method may be expressed by the equation

$$rH = \frac{E_h}{0.029} + 2 \text{ pH}$$

where E_h is the measured potential, and rH represents, roughly speaking, the intensity of oxidation or reduction. Other things being constant, E_h should vary inversely with pH, and this has been verified by a number of workers, for instance, Willis (16) and Heintze (7). By assuming from the above equation that "each unit decrease of pH should be accompanied by an increase of E_h of 58 millivolts (at 20° C)," it is possible to correct any experimental reading of E_h to the value it would have at some particular value of the pH. Pearsall has used the value of pH 5.0 throughout, as being a fairly average value for the soil types he has used, and thus for every sample has a redox measurement (E_s) which is independent of the pH and can therefore be used for comparing soils and mixtures of different types. The method has been applied to a wide variety of soil types in the north of Britain and in Ireland and more particularly to the very moist and water-logged soils of blanket bogs, raised bogs, and lakeside habitats. Misra (8) gives data for actual lake muds, and in the most recent publication (11) a number of results are given for lake waters, and mud and water mixtures, which confirm and amplify the conclusions derived from wet soils.

Pearsall (10) puts forward evidence for believing that the potentials which are being measured really are redox potentials; one strong reason is the general correlation between the potentials and the degree of aeration of the soil. Moreover, the potentials measured electrically are confirmed by the use of indicators, such as

phenol blue, Bindschedler's green, phenol indophenol and various others, which give colour changes at known definite E_h values.

The results show that, as a general rule, completely water-logged soils give E_s values below 200 mV, while the soils of land habitats give values above 380 mV. Within the intervening range must lie most of the conditions which are especially important in considering the species of hydroses and the aeration of their roots.

A very important transition in properties occurs at an E_s value of about 350 mV. This value separates "reducing soils" from "oxidising soils." The former are characterised by the fact that iron, if present, is in the ferrous form. The test for this is a modification of Comber's thiocyanate test for base deficiency, described by Misra (8). If such soils are exposed to air, Fe^{++} becomes converted to Fe^{+++} and the E_h value rises. Moreover, they do not contain nitrates or sulfates, and nitrogen and sulfur are present mainly in the form of ammonia and sulfides, respectively. One exception only to this was found, when nitrates were being supplied to a particular soil by continual influx of nitrate-rich drainage water.

The theoretical equation, quoted above, depends on the assumption that the oxidation and reduction processes are thermodynamically reversible, but this view seems to need modification in view of the results of Cooper (5) and others, which are discussed by Pearsall and Mortimer. Cooper has shown that the observed values for well aerated water (sea-water surface) agree well with the calculated values for irreversible oxidations, especially those that might be concerned in the formation of oxide films on the electrode surface. The observed E_h values which Cooper quotes are mostly much higher than those given by the work on soils and deep water layers, and his calculations suggest that the quantity of dissolved oxygen must be lowered very drastically before "reducing" conditions can possibly occur. Pearsall and Mortimer estimate the necessary concentration at 8% saturation or less.

Correspondingly, their experimental results show a very large increase in E_s (about 100 mV) which can be brought about by bubbling air through their reducing water samples for as short a period as 30 secs. They observed also that the potentials above 350 mV were much more unstable than the lower ones, and attribute this to the effects of the presence of free oxygen.

They suggest tentatively that in water-logged soils, truly rever-

sible systems do occur, but that irreversible oxidations may play a part in determining the potentials, especially in the range where these are unstable.

Numerical data are given by Pearsall and Mortimer which show both redox potentials and dissolved oxygen concentrations for a number of lake water samples. These are given here again because of their importance in considering soil aeration:

Series 1.		Series 2.	
Dissolved Oxygen mgm./litre	E_s	Dissolved Oxygen mgm./litre	E_s
5.1	—	6.35	512
4.8	496	6.0	480
3.6	461	1.10	473
0.3	347	0.8	462
—	290	0.6	350
—	288	0.4	325
		0.4	290
		0.3	280

The first series shows a jump in the dissolved oxygen values, from 3.6 to 0.3; the latter figure probably lies well below the critical concentration for most plant roots, and the corresponding E_s value of 347 is at or below the lower limit of "oxidising soils" so that it might be true that this potential limit corresponds to the limiting oxygen supply for root respiration. The second series, however, goes against this, for it shows that E_s may be as high as 462 mV while the oxygen concentration is only 0.8 mgm./litre.

It is worth turning to some of Pearsall's data on actual plant communities before following up this point. His practical methods of obtaining them are noteworthy, because for the most part he uses soils in their natural condition. Some readings were taken actually in the field by inserting the platinum electrode of the apparatus into the soil to the required depth. Others were obtained from soil samples taken from the centre of large blocks of soil which were transported to the laboratory, thus minimising the changes in temperature and humidity which would affect smaller samples. Some of the readings, it is true, were made on samples treated with toluol, but preliminary tests showed that soils so treated maintained their original potential over considerable periods of time. The point is of importance because most of the previous data on soil redox poten-

tials have been derived from soil samples far removed from their natural state.

The ecological results may be best illustrated by considering three examples which bring out clearly a very marked correlation between pH and E_s values, a correlation distinct of course from that between E_h and pH which allows E_s to be calculated.

Firstly, take the succession from reed swamp to woodland which is seen in regions of fairly rapid silting in places in the English Lake District. The phases are roughly as follows:

- i. Reed-swamp (*Phragmites*) and young *Salix* woodlands on submerged soils with pH between 5.0 and 6.0 and E_s from 0 to 250. The high pH is noteworthy, and it is confirmed for quite a wide range of lake muds, not necessarily belonging to this succession, by the data given by Misra (8), which deal with the subject more fully.
- ii. *Salix* or *Alnus* woodland, with soils varying in pH, but always above 3.8; E_s values below 340 in winter, but sometimes rising higher in summer.
- iii. *Betula* woodland with soil pH always below 3.8, and E_s values of 400 and over.

The data show that the downward drift in pH values over the whole succession does not set in until the E_s values have reached 300, and Pearsall interprets this to mean that below this potential no formation of organic acids is possible.

Secondly, there is the succession from moorland lakes and tarns to raised bogs, characterised by the vigorous growth of *Sphagnum* spp. Here again the early stages of submerged soils start with high pH and low E_s ; in fact, these values are surprisingly like those for the *Phragmites* reed swamp, although the dominant here is *Carex inflata*. The developmental stages, with typical alterations of E_s and pH, are shown in a set of samples from the same lake district locality, Birket close:

	pH	E_s
<i>Carex inflata</i> with <i>Menyanthes trifoliata</i>	5.47	150
<i>Sphagnum</i> spp. with <i>Myrica gale</i>	4.42	170
<i>Sphagnum</i> on pure <i>Sphagnum</i> peat	4.25	280
Bog centre with <i>Calluna</i> and <i>Eriophorum vaginatum</i>	3.82	340
Beside a drain, with <i>Vaccinium myrtillus</i>	3.28	475

The last sample is one of many which indicate the effect of drainage in reducing the pH, which for an untouched raised bog does not reach extremely low values.

Lastly, we may consider a contrast which can be seen between the blanket bogs of Connemara in Ireland, with much *Molinia* and some *Sphagnum*, and the Cotton Grass moors of northern Britain, which are in fact a type of blanket bog. The former show pH values of 4.4 to 4.6 and E_s values probably below 300, whereas the latter have E_s values over 300 and pH values round 3.3. A suggested explanation lies in the difference in annual rainfall—85 to 120 cm. for Connemara, 60 to 80 in the *Eriophorum* region. The soil, at any rate the surface soil, in the drier region more often emerges above the water table and hence can develop acid conditions which are tolerable to *Eriophorum* but not to the common blanket bog species. Acid though the *Eriophorum* soils are, still more extreme conditions are reached in the redistributed peat habitats which result from the retrogression of the continuous *Eriophorum* cover. On these peats *Empetrum nigrum*, *Vaccinium Myrtillus* and *Calluna* come in; pH values of 3.0 and E_s of 400 and over are recorded. The species just mentioned differ from *Eriophorum* in that their roots do not possess extensive internal air-spaces, and Pearsall considers that his results support the view of Moss (9) that such species indicate a soil which is not oxygen-deficient, in contrast to that occupied by *Eriophorum*. If this is so, it would seem that the redox potential that separates an "oxygen-deficient" from an "oxygen-sufficient" soil should be in the region of E_s 400. But there are difficulties about accepting this conclusion even tentatively. In the first place there are the results of Pearsall and Mortimer, already quoted, which show that an E_s value of well over 400 mV may be associated with very low oxygen concentrations. In the second place, and arguing in the reverse sense, there are species which grow in the bog soils of the *Sphagnum-Myrica* type, for instance, with E_s below 300, whose roots do not seem particularly adapted to an "oxygen-deficient" medium. *Myrica* itself, *Erica tetralix* and *Andromeda polifolia* may be mentioned as examples.

Evidently, therefore, as they stand, the redox potential results do not enable us to distinguish the soils which demand an "aerating mechanism" of the *Cladium* type, from those which do not, although they are very suggestive. It seems that more data are needed as to:

1. The correlation between oxygen content and redox potentials under different conditions.
2. The critical oxygen concentrations for the root supply for individual species.
3. The type of root in different species and the distribution of roots in the soil. Pearsall's E_h measurements were taken at 10 cm. depth in the soil, and possibly species such as *Erica tetralix* have many of their roots in the very uppermost soil layers, thus evading the more reducing condition lower down. Again, in any given habitat the soil may be water-logged for part of the time only, and plant roots may survive these periods if they are short enough, even though the species would not tolerate permanent "oxygen deficiency."

One has also to remember that redox potentials themselves may be of importance in root respiration, apart from their use as indicating oxygen concentrations—in so far as they do indicate them. Remembering too how plants may be indirectly affected by reducing conditions, through nitrate lack or toxicity of ferrous ions and so on, it is clear that a great complexity of factors is involved in the ecology of helophytes and "amphibious" plants in general. An illustration of this can be seen in the conclusion which emerges from Pearsall's data concerning *Molinia coerulea*, namely, that it will inhabit only reducing soils provided the pH is above 4.4, or oxidising soils provided the pH is below 3.9; as Pearsall puts it, "high acidity may be supportable only when oxygen is present." Though it does not seem possible at present, it is very desirable to give some account of the meaning of this statement in terms of the plant metabolism, and it is not until we have acquired and interpreted such information for many different species that we shall be very much further forward with the question of plant growth in wet soils. Nevertheless, the methods of investigation are there, and have proved their usefulness; their further application should yield results of much value.

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AMITOSIS

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It is probable that next to the basic question of the origin of life a majority of biological specialists would place as second in importance the problems associated with reproduction of the species; and our understanding of reproduction, after all, is largely dependent upon our knowledge of cell division, the basic multiplicative process.

Those who have worked upon the subject of cell division can well be proud of the present factual state of our information concerning it. There is much that is definitely known, that is incontrovertible. However, there are still some points where agreement among authorities has not been reached, and where our data are so incomplete that even if there were concurrence the ideas would still be too subjective to merit classification among the solved problems.

In view of the relatively objective and approachable character of cell division and its great theoretical importance it is to be lamented that of late years so little effort has been made to round out our knowledge. This is especially true of amitosis. To a large extent investigation of it has lapsed. This is probably due both to seeming difficulties of study and to a belief among some students that the status of amitosis is settled. The latter view is not tenable so long as disagreement is rampant.

THE PROBLEM OF AMITOSIS

The word *amitosis* implies merely an absence of mitosis. The latter word is derived from the Greek *μίτος*, meaning thread. Accordingly, our use of it to signify the division of the cell accompanied by a thread formation in the nucleus is a logical one. Originally, then, amitosis was a synonym for *direct cell division* which was interpreted as division of the cell by constriction following constriction of a resting nucleus.

For some years, however, it has been evident that amitosis must be considered under three heads, namely, a constrictional division of the nucleus not associated with cytodieresis, or fission of the cell, the problematical one in which also the cytoplasm supposedly divides, and the division of the ciliate macronucleus.

There is essentially no difference in the degree of confusion or the ultimate question relative to amitosis in the plant and animal kingdoms, so the two can best be considered jointly. In the following discussion observations will be drawn from the kingdom best suited for illustration of the point at hand.

AMITOSIS AS A NON-REPRODUCTIVE PHYSIOLOGICAL PROCESS

No matter what view one may take of the possible interchangeability of mitosis and amitosis, all are agreed that in some tissues there is regular amitotic division of the nucleus that is never followed by fission. One of the clearest cases is to be found in the hepato-pancreas of the crustacean *Porcellio*. The number of cells in the organ is never increased once maturity is reached. With the attainment of this stage all the cells become binucleate through amitotic division of the nucleus. The complete absence of any further cell division, in fact, the maintenance of a constant number of cells, permits the dogmatic conclusion that in the hepato-pancreas of *Porcellio* amitosis invariably leads to a binucleate state and that the process is non-reproductive so far as multiplication of cells is concerned. The diagrammatic clarity of the material (whole mounts lightly stained with Delafield's haematoxylin are adequate for demonstration of the process) has led some to the view that all of our ideas of amitosis should be based on similarly perfect subjects for investigation and has made them discount studies on the mammalian liver or muscle which point to other conclusions.

There are numerous identical instances, a few of which may be cited. Malpighian tubules of the cricket become binucleate in the same manner. The follicle cells of the cricket afford ideal material even for classroom study of this form of amitosis. Once the tissue is hardened the one cell thick follicle may be chipped off, stained and mounted *in toto*. By selecting pieces from mature eggs, medium sized and small ones, it is possible to obtain the series of binucleate cells, cells in which amitosis is occurring and uninucleate cells.

It is necessary to enter the realm of conjecture in order to assign a purpose to the process. This will probably continue to be true because of its unapproachability by experimentation. One of our best clues is to be found by comparison of the Malpighian tubules of the cricket and spider. The former are binucleate, the latter

greatly lobulated. The one common result of the two occurrences is increase of nuclear surface. This suggests that in highly specialized cells, which genetically have no future, efficiency or capacity in vital activity is improved by increasing nuclear surface, which may be accomplished by amitosis.

Nothing has been contributed to the study of this type of amitosis for a number of years. The above brief résumé is given to form a background for evaluation of recent articles dealing with reproductive possibilities of amitosis. [For a more thorough discussion of this so-called physiological amitosis refer to the paper by Conklin ('17).]

AMITOSIS IN THE CILIATE MACRONUCLEUS

Of the two nuclei of the typical ciliated protozoan, one, the vegetative or macronucleus, always divides amitotically, while the other, the micronucleus, is duplicated by mitosis. This is true at every simple fission. And this constitutes the one unquestioned instance of cleavage of the cell following amitosis. However, from the theoretical viewpoint it is hardly comparable to amitosis in any other organism, nor can it serve as an establishment of the sufficiency of the process as a method of cell division, because the macronucleus is not permanent. Though we still do not understand the exact relationship of the two ciliate nuclei, the micronucleus is the one which must be regarded as genetically comparable to that of other cells. It is permanent. The macronucleus is a transitory one whose fate is degeneration, at which time it is replaced by a product of the micronucleus.

The commonly accepted picture of macronuclear amitosis is simple division by dumb-bell formation in which there is no obvious internal activity or preparatory reorganization. This is not universal. Turner ('30) has thoroughly described a reorganization process in *Euplotes* which is more complicated even than mitosis. Beginning at the ends of the long horseshoe-shaped macronucleus the chromatin reticulum discharges the disperse achromatin from the area, forming a solid terminal band of chromatin. These bands move towards each other, obliterating the visible diphasic character of the nucleus. In their immediate wake slender parallel threads of chromatin traverse a continuous substratum of achromatin for a distance equal to the width of the band. Beyond this the threads seem to spread in three dimensions and to join to form a continuous

reticulum indistinguishable from that beyond the band. The nucleus shortens during the reorganization. When the bands meet, cleavage occurs between them. Even though this is not the usual method, the fact that such a visible reorganization is present in isolated cases should caution us not to be too ready to regard amitosis as a mere quantitative fragmentation in any instance except the established physiological amitosis.

AMITOSIS AS A REPRODUCTIVE PHENOMENON

Unlike the *Porcellio* material, all descriptions of amitosis as a method of cell division have been from tissues which for one reason or another do not readily lend themselves to the study. Thus we find the protagonists of the idea drawing illustrations from the flagellated Protophyta and Protozoa, the rhizopods, yeasts and highly specialized tissues such as liver and cartilage of higher animals and endosperm of flowering plants.

In the flagellated protistan the source of confusion is the difficulty of distinguishing amitosis from the crowded intranuclear karyokinetic figure, as well as from the fact that the organisms are rather hard to fix and stain properly. The rhizopods have the same handicaps.

In yeasts the trouble lies not so much in the nucleus as in the character of the cytoplasm. An abundance of basophilic cytoplasmic granules either obscures the nuclear figure or makes interpretation impossible. The significant character of this source of error will be realized when we recall that it was the same thing which in former years led so many to the belief that yeast did not have a centralized vesicular nucleus.

Thus, in the Protista the disagreement is as to whether or not the nucleus divides amitotically. With a few exceptions this is not the problem in higher organisms. All agree that numerous nuclei divide amitotically. One group believes that this is followed by cytodieresis, the other contends that it merely produces multinucleate cells. In other words, they say it is the same physiological amitosis as found in *Porcellio*.

Though it would not be applicable to every investigator, it is generally true that those who have worked upon the clear-cut material in which the conclusion that it is physiological amitosis is inevitable, take the second view and deny or doubt cytodieresis;

while those who have depended solely upon specialized tissue of higher organisms favor interpreting amitosis as a method of cell division. Although at first thought it would seem that we are dealing with the simple objective problem of whether or not fission follows amitosis the difficulty of observing the process microscopically is such that the whole matter is still in a subjective state, and opinions naturally differ.

It is also probable that the general reaction to amitosis is somewhat controlled by the imagined effect of its acceptance on the chromosome theory of heredity. This is unfortunate. It is nothing more than a matter of using a standard of truth in situations where there is not good indication that it applies. As a matter of fact, acceptance of direct cell division would confuse our concept of heredity little more than the well established chromosome aberrations in germ cells. But even if there be any inconsistency between amitosis and the chromosome theory of heredity, our problem is still to bring the objective aspects of fission within our objective view, and cross the bridge of conflict when we arrive there.

Because of the basic differences in the causes for disagreement in the various groups of organisms they can best be considered separately.

Protozoa. Protozoologists have altered their view of amitosis more in the past half century than any other group of cytologists. From a state of favor of amitosis or a pseudomitosis, so simplified as to be hardly classifiable as mitosis, they have in general come to approve the view expressed by Kofoed ('23) in these words: "In the first place amitosis as described in the Protozoa is either a pathological or degenerative process, as it is in the Metazoa, or it is based on a partial account of the normal process of mitosis in which the nuclear membrane remains intact throughout the whole process, as it does in the flagellates and rhizopods, and in its anaphases presents a superficial resemblance to pathological amitosis. The persistence of the nuclear membrane in no way interferes with the occurrence of chromosomes constant in number and kind. In other words the doctrine of chromosome continuity, in so far as amitosis is concerned, is no more affected in the Protozoa than in the Metazoa." Thus, refinement of technique and careful observation have surmounted the difficulties which once placed many Protozoa on the lists of amitosis.

It will be noticed that Kofoid leaves open the possibility that amitosis may serve a reproductive purpose in pathological specimens. Several later papers reflect significantly upon this. Wenrich ('24), in a study of the euglenoid *Euglenamorphs*, found a degeneracy very common which involved principally the loss of chlorophyll. In the green individuals all nuclear division was mitotic; in some colorless ones he observed amitosis. Nor was it a confused picture. There was a clear-cut resting reticulum all during constriction. It is important to note that he did not observe fission of the cell, not even an initial furrowing. However, he considered it plausible that fission might follow amitosis in these pathological specimens.

Kater ('30) has described an amitosis in the cryptomonad *Chilomonas* that could be followed much nearer completion. When chilomonads are enmeshed in the zoöglea of their culture they sometimes become obviously degenerate. The typical shape is lost and they attain a much larger size than is normal. In this state not only does the nucleus divide amitotically but the cell becomes furrowed also. Numerous individuals were found in which the furrowing was almost completed. A number of these were selected and watched continuously under the microscope. Invariably the furrowing continued until it seemed the time had come for daughter cells to separate. Then cytolysis would occur. It was concluded: "No protozoologist is justified in doing more than suggesting, as a slight possibility, that amitosis is followed by fission, even in pathological states, unless he has observed fission proceed to completion in living organisms."

This observation cannot yet be generalized, and we must hold open the possibility that amitosis may be multiplicative in some pathological states of Protozoa. But there is not a single instance that fulfills the requirements of the above quotation, so it is merely a possibility, not a probability. Although there is no recorded case of definite physiological amitosis in Protozoa, or of any amitosis of the generative nucleus, in normal individuals the amitosis in *Euglenamorphs* would seem likely to afford a pathological counterpart of it.

Yeast. Unfortunately there is no comprehensive treatise or expression of a consensus of opinion regarding amitosis in yeasts with which we can start. One of the first investigations in which the

nucleus of this plant was clearly recognized contributed the opinion that yeast divides by a simplified mitosis (Janssens, '02; Janssens and Leblanc, '89). Closely following this another pair of investigators described the division of the yeast nucleus, but, unfortunately, to them the nucleus included the actual nucleus, the glycogen vacuole and the cytoplasmic metachromatin. In view of this we can hardly attach weight to their conclusion that nuclear division is amitotic in the case of budding and an "intermediate step in karyokinesis" in spores (Wager, '98; Wager and Peniston, '10).

After an extensive series of studies on yeast, Guilliermond ('04, '12, '17) was of the opinion that the division is amitotic when buds are being formed, and a true mitosis in ascospore formation. It should be emphasized that should this view be substantiated it would make mitosis and amitosis more or less equivalent and interchangeable because each would occur in the same plant at different points in the life cycle. In other words, a cell produced by amitosis would be capable of a later normal mitosis.

Swellengrebel ('05) and Fuhrmann ('06) have described the division as true mitosis. The paper by Fuhrmann especially gives the impression of being carefully and accurately done.

On a basis of these contributions it would seem that there is no question about division in the ascus. It is mitosis. Regarding budding the opinion is rather evenly divided. In this state the subject was permitted to come to rest. This was just about the time that the protozoologists were dispelling the last shreds of evidence for amitotic division in normal Protozoa.

It cannot be said that there has been any real resumption of interest in this problem of yeasts, even at the present day. In the past two decades only two articles dealing with the subject have appeared (Kater, '27; Badian, '38). Both of these maintain with utmost certainty that the division is mitosis. It must be admitted that they swing the balance toward that as a general acceptance, but for specific reasons they do not justify the complete discard of the contention of Guilliermond, based as it is on such prolonged investigation of the cytology of yeasts.

For an unbiased critic there is one feature of the work of Kater which somewhat discourages generalization. The technique of preparation was admittedly dependent to a certain extent upon accident. The slides were made by staining cells containing picric

acid with iron-alum-haematoxylin. Since picric acid is a destaining agent for haematoxylin the material was not permanent. The value of the method rests upon the fact that in the presence of picric acid the nucleus will stain before the cytoplasmic granules, but the required balance of dye and acid appears to be quite delicate so that duplication of results is difficult. Although the clear-cut cells on the slides were very convincing to an actual observer, it can hardly legitimately be the basis for a general acceptance of the conclusion by all workers in the field until others manage to duplicate the results.

The one later paper, that of Badian, does not fill the possible leak in Kater's work, although the general conclusion is the same, because his duplication of idea did not depend upon duplication of technique. In addition, Badian's work is not sufficient in itself, for two reasons. In the first place, he draws a very close parallel between the cytology of bacteria and of yeast, which is not justified on a basis of present knowledge. Secondly, his figures and description, particularly of the resting nucleus, are suggestive of somewhat inadequate technique in preparation. However, this last criticism could equally as well be applied to his principal opponent, Guilliermond.

To conclude the evaluation of amitosis in yeast, we can say that it does not occur in the ascus. The method of nuclear division at the time of budding is not established, but the greater part of the evidence favors mitosis. Though it is very doubtful if amitosis occurs at all as a reproductive phenomenon in yeasts, the burden of proof still rests with both sides.

Specialized tissues of higher organisms. This phase of the subject can properly begin with the reviews of Conklin ('17), Tischler ('22), and Sharp ('26), the first drawing principally upon animals for data, the latter two on plants. All had before them the same panorama, yet their conclusions were at variance. This fact shows clearly that more work is needed on this supposed type of amitosis.

Tischler admitted that amitosis may be reproductive in some cases, though he would greatly restrict the likelihood of its occurrence. Sharp would probably restrict it somewhat more. Conklin denied all possibility of its ever being multiplicative and showed that even experimental or pathological divisions are basically mitotic. He said, "Mitosis and amitosis are fundamentally unlike.

Mitosis is the one and only method of bringing about equal division and distribution of the chromatic material of the nucleus. Amitosis is not a genuine divisional phenomenon at all, but merely a means of increasing the nuclear surface and of distributing nuclear material throughout the cell, comparable to nuclear lobulation, fragmentation or distribution. These two processes are not equivalent or even comparable, nor may one of them be converted into the other."

These three reviews gave a very fair picture of the contentions that had appeared in the literature. But the conclusions hardly did justice to the prevalence with which at that time amitosis was regarded as reproductive. The moderate view of Tischler and Sharp had perhaps the greatest following; the sweeping denial of reproductive possibilities of amitosis by Conklin, incidentally one of the most dogmatic generalizations ever made in biological sciences, expressed the view of one minority; and still another minority, not spoken for, in the conclusions of these papers, rather definitely held amitosis to be reproductive.

Before evaluating the late contributions individually, it is well to have clearly in mind the arguments, pro and con, which made Tischler, Sharp, and Conklin so skeptical of the amitosis thesis in spite of the voluminous favorable literature.

It has already been mentioned that all agree to the fact of amitotic division of the nucleus in specialized tissues of higher organisms. The dissension, then, is wholly in regard to cleavage of the cytoplasm. At the time the above reviews were written it was admitted that this could not be satisfactorily demonstrated. The pro side argued that physiological regeneration must occur, that mitotic figures are not abundant, or are even lacking, while amitosis is very common. Therefore, they said, there is cell division, and amitosis is the only observable source of nuclear division.

The con side said cytodieresis is not demonstrable after amitosis, while it is easily demonstrable, even in crowded cells, after mitosis. If cleavage occurs, then demonstrate it. These were the most emphasized points in both arguments.

In yeasts neither mitosis nor amitosis is generally recognized as established in the case of budding. As stated above, this puts the burden of proof on both sides. In somatic tissues of higher organisms this is not true. Mitosis is admittedly a possible method

of cell reproduction. Therefore, the burden of proof rests upon the advocates of amitosis. And the following papers fall definitely short of supplying that proof. It is to be noted that though most of the late work has been done on animals the only papers which establish their contention with complete satisfaction are those of Smith and Cooper on plant material, to be discussed chronologically in the ensuing pages.

Komuro ('32), after a cytological study of coal tar tumors in root-tips of *Vicia faba*, concluded that "It appears" that the growth of the tumor is due to an amitotic multiplication of the cells. It is obvious that he was not entirely convinced, or his conclusion would have been stated more definitely. Shortly after treatment the cells were so affected as to make them stain evenly and deeply with basic dyes. Later, as this quality was lost, the cytoplasm became greatly vacuolated, and Komuro suggested a mechanical relationship between this process and the fragmentation of the nucleus. In many cases a great number of nuclei were formed, and in some instances these seemed to fuse to form again a single nucleus. The formation of a cell plate could not be followed with certainty.

Smith ('33) and Cooper ('33), both working on tapetal cells, agree that all nuclear division is mitotic. These two papers are extremely convincing. Both the figures and description are clear-cut, and Cooper advanced observations which satisfactorily explain the semblance of amitosis which is presented by some tapetal nuclei. He found that non-disjunction of anaphase chromosomes may create a bridge between the reconstructing daughter nuclei. These connecting chromosomes undergo the same telophasic changes as the daughter nuclei so that a single resting nucleus in an amitosis-like dumb-bell is produced. This contention is substantiated by a well selected series of illustrations. Additional weight is given by the fact that twenty-four families are represented in the material studied.

On a basis of this work it seems justifiable to conclude that the amitosis problem is solved by negation so far as the tapetum is concerned. This is especially significant since in normal instances the divisions are only physiological, not reproductive. It is also interesting that this bulk of data supports the very early suggestion of Strasburger regarding the seeming amitotic dumb-bells of the tapetum.

MacMahon ('33) resurrected the old favorite for studies on amitosis, the liver. He concluded, as have others before him, that mitosis and amitosis are interchangeable. Several aspects of his work introduced definite departures from earlier papers. He believed that failure of fission is as likely to follow mitosis as amitosis. In other words, some of the binucleate liver cells arise by mitosis. Also, he attempted to strike at the crucial point in the amitosis controversy. His paper is illustrated with photomicrographs, and he included pictures which give the appearance of cleavage accompanying amitotic division of the nucleus. He considered these to be absolute proof of the reproductive character of amitosis.

It must be admitted that the pictures appear to prove his point. However, analysis of the general tissue portrayal indicates poor fixation, and interpretation of a photomicrograph is, at best, difficult because of the ease with which diffraction of light can confuse the picture. In addition, most of the scientific work of this reviewer has been devoted to the liver, and there is little similarity in the fine points of architecture of the liver cells as seen in his pictures and in actual slides. Above all, he did not give a detailed description of the exact technique employed in the preparation of the slides.

A view similar to MacMahon's was advanced by Clara ('35) on a basis of the kidney tubules. However, Clara depended solely upon the old argumentative basis and did not claim to have seen cytodieresis. In young tubules he found abundant mitosis and no amitosis. In old tubules there was little mitosis and abundant amitosis. He argues that physiological regeneration must be occurring; therefore, amitosis is reproductive.

It seems probable to this reviewer that much of the prejudice on the part of many cytologists against the amitosis thesis of Clara and his predecessors, and the consequent dogmatism with which they deny the possibility of reproductive amitosis, has arisen because of their reaction to the persistence with which this perfectly inane and senseless argument has been stated and restated. Clara admitted the occurrence of occasional mitosis. He made no calculation of the amount of physiological regeneration that could be traced thereto. In fact, he had no way of knowing how much for which to account. Yet he concluded that the mitosis observed was not

adequate to effect the regeneration, and further stated that we must consider amitosis to give "full value and livable" cell division. The general conclusion, unrelated to the evidence, becomes even weaker as Clara observed that in pathological regeneration only mitosis is found when growth is rapid; when it is slower, both mitosis and amitosis occur. He concluded that both were reproductive. Finally, he expresses wonder that mitosis should take place when amitosis is adequate for all purposes of the cell.

It is to be remembered, however, that the fact that so many have rested their case on this type of argument does not justify the discard of the possibility of amitosis.

The work of Stough ('35) offers a pleasant contrast. Although he denies the existence of a true amitosis he supports the occurrence of what most investigators have termed amitosis. He says, "As far as the chick, and probably all vertebrates, are concerned it is likely that there is no such process as amitosis," and again, "Modified mitosis is not amitosis but is in many cases in all probability what others have reported as such."

The cytological details of his analysis of the nuclear division (modified mitosis or amitosis) will be discussed in another section. At present our concern is only the relation of his data to cell division. Like MacMahon he perceived the futility of mere guesses. His approach was different. He painstakingly counted the number of mitotic figures, and using the best available data on the duration of mitosis attempted to calculate the possible growth, in comparison with the actual growth rate in several tissues of the developing chick. Actual growth was much greater than the calculated possible growth based on the number of mitotic figures. Because of its lack of dogmatism and its moderate character the following conclusion seems justified: "A mathematical study of cell division in the chick concerning itself with cell counts and mitotic indices indicates that typical mitosis does not occur in sufficient quantity to account for growth. As all calculations are made using a mitotic interval which is undoubtedly very much too low, the writer feels that these results are conservative. Although they are not presented as absolute proof, still they are consistent, and taken as a whole, point in the direction of the truth and constitute very strong evidence that modified mitosis is a process of prime importance in the growth of the embryo and that it is impossible for typical mito-

sis longer to maintain its position as the exclusive source of new cells."

Elliot ('36) has duplicated the work and conclusion of Clara, using cartilage. He concluded, though somewhat less dogmatically, that amitosis is proliferative. His argument was the same as Clara's. He said, "In the first place it can hardly be doubted that wear and replacement occur in normal articulations and failing mitosis some other process must provide this replacement." He also claimed to have seen evidence of cleavage by ingrowth of the matrix. However, though the paper is well illustrated otherwise, this one critical stage is completely omitted.

Marquardt ('38) reported that X-rays sometimes caused non-disjunction of anaphase chromosomes in plants, and that ensuing reconstruction forms dumb-bells similar to those described by Cooper ('33). He calls this "Pseudoamitosis."

Loreti and Perroncito ('38) observed that experimental pilocarpine fatigue of the parotid gland led to the production of giant cells whose nuclei underwent amitotic division. They found no indication that the cells themselves ever divided.

In the earlier days of tissue culture that technique naturally gave hope that it could be utilized to solve the amitosis problem. And for years the work of Macklin has been quoted as largely fulfilling the optimism engendered by tissue culture. Macklin's conclusion was that amitosis is non-reproductive and that upon the division of an amitosis-produced binucleate cell the two nuclei fused and then underwent normal mitosis. Lately, however, Gatenby and Hill ('34) have studied the problem in cultures of tissue from *Helix* and reached the opposite conclusion. Though the material is not clear enough to justify dogmatism, they definitely favored interpreting this tissue growth as a result of amitosis. These directly contrary conclusions in two papers, both of which have the requisites which demand respect, suggests that tissue culture introduced difficulties of its own as regards observations on amitosis. It does not seem likely that tissue culture will afford the proper approach to amitosis.

STOUGH'S ANALYSIS OF AMITOSIS

Except for the reference to *Euplotes*, all of the preceding has dealt with the cell reproductive character of amitosis. It has ac-

cepted the nuclear phenomenon as a simple unanalysed fact. Actually, it is surprising that the literature has done this for so long. In clear cases of physiological amitosis in Metazoa the recorded observations are adequate to permit an accurate description of the cleavage. It is a simple constriction of a resting nucleus that is indistinguishable from all other resting nuclei in the tissue. In the controversial cases of amitosis there has been too much of a tendency to apply the same description without checking the material at hand.

Stough has conducted a prolonged study of nuclei in amitosis (modified mitosis) and has compared them to other resting nuclei. He has been led to the belief that the two are definitely not the same. Further, he sees evidence of an orderly internal change rather than a simple cleavage. It is this which has caused him to suggest that the division should be called "modified mitosis," rather than amitosis. His description can well be quoted: "Modified mitosis is primarily a nuclear phenomenon. Metaphases, with their conspicuous arrangements of chromosomes are not present. Neither can centrosomes be found. Instead, the chromatin, in the form of a conspicuous, deeply staining nucleolus-like structure elongates, constricts to form a dumb-bell shape, and finally pulls apart into two masses. These latter, since they appear not to be homogeneous but to be composed of small particles closely clumped, have been called 'mulberries' (figs. 1, 2). Between these may be seen structures which may represent remnants of spindle fibers. Following chromatin division a very fine membrane or nuclear plate may be seen forming which later divides the nucleus into two daughter nuclei. Very rarely has constriction been observed taking the place of nuclear plate formation. During this entire process the nuclear membrane does not disintegrate as in typical mitosis."

An evaluation of Stough's work is not at present practical. However, it may be said that it is suggestive, and whether it proves true or not the minute details of resting nuclear structure or modified mitotic structure which he has studied in something of a statistical manner reminds us that much of the minutiae of the nucleus is yet to be adequately described. It might also be said that in view of the clearly observable profound nuclear changes during amitosis of the macronucleus in *Euplotes* (Turner, '30), it is not inconceiv-

able that more observations and a greater variety of techniques may disclose a more organized process than is commonly assumed in other cells.

Obviously this phase of the work in no wise affects the question of the reproductive nature of amitosis. That remains the same whether the nuclear division be called modified mitosis or amitosis.

OMISSIONS

It will be noted that in the above review the blue green algae and bacteria are not mentioned, in spite of the fact that work has been done on their division. The question of the character of the division of the material corresponding to the nucleus of other forms, is, of course, important, and must some day be directly related to mitosis. The author considers that it would be futile to attempt this at the present time. The analogies of interkinetic structure are not yet definitely known, and this step must be taken first before the problem of division can be discussed, even though it may be desirable for the two studies to be pursued concurrently.

Several late efforts to compare the bacterial nuclear material with the vesicular nucleus may be mentioned. Badian ('37) believed the bacterial nucleus to be identical with that of the yeasts. Hollande ('37) introduced an extremely intriguing approach. Instead of taking the vesicular nucleus as the standard and comparing the blue greens and bacteria to it he formulated definite concepts of the cyanophycean type and then tried to analyse the individual elements of the vesicular nucleus in terms of the blue green structure.

SUMMARY AND OUTLOOK FOR CLARIFICATION OF THE AMITOSIS PROBLEM

1. In some instances amitosis is unquestionably a non-reproductive process, probably necessitated by the specific functions of the tissues involved.

2. Amitosis is clearly reproductive only in the non-genetic macro-nucleus of the ciliate.

3. The so-called simple division of the normal protozoan nucleus is not amitotic.

4. That pathological amitosis of Protozoa is ever followed by cytodieresis is very questionable. Definite solution of the prob-

lem can be expected because all that is required is for those finding such nuclear divisions to watch similar specimens and see if they divide.

5. Division in the yeast ascus is not amitosis.

6. Division of the budding yeast cell is more than likely not amitosis. Definite word on the question should be forthcoming. Skillful application of the sometimes deceptive nuclear reaction should be very helpful.

7. The status of the amitosis question in highly specialized tissues of higher organisms has not been greatly altered in the past two decades. Opinion is still divided into three groups: (a) those maintaining that all amitosis is physiological and non-reproductive; (b) those believing definitely that it is reproductive; and (c) those who leave the matter as still open.

8. If the observations of MacMahon and Stough were wholly accredited and their interpretations accepted, the reproductive character of amitosis would be established.

9. The statistical approach followed by Stough in trying to determine if mitosis were adequate to account for growth may, if followed sufficiently far, contribute to a solution of the amitosis problem.

10. Search for cytodieresis (MacMahon), the critical point in the controversy, could be regarded more hopefully if approached by use of techniques of fixation designed to accentuate cell boundaries, rather than conventional histological methods. There is no reason why we cannot hope for a solution on this basis.

11. Without a great change of procedure it seems unlikely that tissue culture will afford a good approach to the reproductive possibility of amitosis.

12. The tapetum, ordinarily classed as a case of physiological, and occasionally reproductive, amitosis has been definitely placed in the mitosis category.

13. The division of Cyanophyceae and bacteria cannot yet be profitably compared to that of other forms.

14. Though the data do not permit a definite conclusion this reviewer doubts that amitosis will prove to be a method of cell reproduction.

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ENVIRONMENTAL INFLUENCE AND TRANS-
PLANT EXPERIMENTS

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One may approach the study of the effects of environment upon plants from one of several viewpoints. Physiologists aim to determine the effects of one external variable at a time in order to discover something of the plant's mechanism. Geneticists are concerned with the expression of gene characters under different sets of conditions. Ecologists and taxonomists usually think of environment in terms of natural habitats which, of course, involve whole complexes of biological and physical factors that interreact.

We shall restrict this review to certain aspects of the latter viewpoint, namely, to experiments designed to determine the effect of transplanting wild plants from one natural environment to another. Here the principal questions at issue are: What kind of changes take place? How do these changes affect plant classification and concepts of evolution?

It is generally agreed (2, 18, 21, 22, 26, 29, 38, 43, 75, 81) that most species are not simple units, but consist of assemblages of interrelated forms having common characters by which they may be identified. Within a species, smaller subdivisions may be clearly enough differentiated to warrant a name, but the number of distinguishable forms is usually so great that no system of nomenclature can possibly describe each variant.

Yet complex as species composition may be, it is not disorderly. Certain characteristic types are usually found in certain kinds of habitats; indeed, plants reflect their surroundings so intimately that some biologists still contend that they are molded by the direct influence of their environment, and that acquired characters become fixed in heredity. Here we meet the age-old controversy between the Lamarckian and the genetic viewpoints. The evidence now available seems to point to a solution.

EFFECTS OF TRANSPLANTING TO DIFFERENT ENVIRONMENTS

Many common species grow over a wide range of natural conditions, and have distinguishable forms associated with different habitats. Such species may extend from the seacoast inland, from a region of heavy rainfall to arid country, from a southern latitude northward, or from a low altitude to a high one. The plants occurring at the extremes of the range are usually the most unlike, while those from intermediate situations may form intergrading series with the extremes. If a race is transplanted from one habitat to that occupied by another related one, will it become like the form native to the new environment? If it remains distinct, does it undergo any morphological change, and if so, what is the nature and significance of this change?

Kerner (50, 51) was one of the first to investigate these problems. Between 1875 and 1880 he established an alpine garden in the Tyrolese Alps at an altitude of 2195 m., and utilized the botanical gardens at Innsbruck, 569 m., and at Vienna, 180 m., as lowland stations. He grew over 300 species of perennial plants from seed at the contrasting altitudes and compared them over a period up to six years. Kerner found that his plants retained their original specific characters after transplanting, but each was modified to some extent by the change in its environment. Thus, lowland plants brought to the alpine station became lower in stature, had smaller leaves, shorter internodes, smaller inflorescences, fewer flowers, and more brilliant floral tints.

While these changes tended to make the lowland plants resemble superficially the dwarf forms native to higher altitudes, transplanting did not affect their fundamental structural characteristics. It did not transform lowland into alpine races, for the alterations were merely quantitative and within definite limits characteristic of the species. Moreover, lowland races, which are slower in seasonal development than alpine, did not often mature at the high altitude. Only 32 out of 300 species were able to develop flowers during the short growing season at the alpine station. In a few cases, species from low elevations developed ripe seed at the high altitude. These Kerner harvested and sowed at the botanical gardens, where they developed into plants indistinguishable from those which had been kept at Vienna or Innsbruck. Kerner also experimented with annuals, and found that species originally from the lowlands were

generally unable to survive at the alpine station. Only a few reached the flowering stage, but stems and internodes on these were shorter and inflorescences less extensive than on the same species grown at Vienna or Innsbruck. Some which failed to flower at the alpine station wintered over and developed new shoots the following spring. Thus, Kerner reported that *Poa annua* L., *Senecio nebrodensis* L., *Viola tricolor* L., and *Cardamine hirsuta* L., among other annual species, became perennial in his garden in the Tyrolean Alps.

His investigations led Kerner to conclude that there are two kinds of characters in plants, those which are modifiable and those which are constant through heredity. He attributed hereditary characters to the structure of the protoplasm, while the relations between it and the external environment determined the inconstant or temporary modifications. The reader will recognize a similarity between this view and the current concepts of genetics.

A very different interpretation was given by Bonnier (7, 8, 9) to the results of his much-cited experiments. Between 1884 and 1889 Bonnier planted 203 cultures of various species of perennials at different altitudes in the French Alps and Pyrenees. He chose groups occurring over a wide altitudinal range having distinguishable forms at different levels. Usually lowland and alpine forms corresponded to subspecies and varieties of the same species, but in some cases related plants, generally accepted as different species, were involved in an experiment. Whenever possible, individuals were dug at an intermediate elevation and divided into two or more vegetative parts. One of these was moved down the mountain to a lowland station, while the other was taken to a higher altitude. Soils also were moved in an attempt to keep the edaphic factors constant. Bonnier moved a few alpine plants to low altitudes, but transplanted a far greater number of lowland forms to higher elevations.

Gardens at Mirande (in the Department of Gers), at Paris, and at neighboring points served as low-altitude stations both for the transplant series in the Alps and those in the Pyrenees. In the Alps, stations were established on the Mount Blanc chain at l'Aiguille de la Tour at 2300 m., at points just below the glaciers, and in the subalpine zone near Chamonix at 1060 m. Other transplants were variously placed in rocks and declivities at intermediate

points. In the Pyrenees, plantings were made in the alpine zone at Col de la Paloume (2400 m.) and at a submontane station at Cedéac (740 m.). Some cultures were also distributed at la Hourquette d'Arreau (1520 m.), at Col d'Aspin (1500 m.), and other places in the drainage basin of the Arve.

The cultures established between 1884 and 1889 were observed by Bonnier until 1919, his results being summarized in three memoirs (7, 8, 9). The most striking result which he reported was the gradual but complete transformation of lowland forms into alpine types (9). In the course of eight to thirty-five years, depending upon the species, Bonnier claimed that the contrasting forms gradually became morphologically and anatomically indistinguishable. Examples of conversion of one species into another include *Helianthemum vulgare* Gaertn. which became like *H. grandiflorum* DC., *Polygala vulgaris* L. like *P. alpestris* Rchb., *Silene nutans* L. like *S. spathulaefolia* Jord., *Silene inflata* Sm. like *S. alpina* Thomas, *Lotus corniculatus* L. like *L. alpinus* Schleich., *Solidago Virgaurea* L. like *S. alpestris* W. & K., to mention only some of the cases. Much more frequent were the reported conversions of lowland forms into alpine types whose differences were merely of a subspecific nature. Bonnier also found that certain lowland annual species became perennial when grown at high elevations.

Bonnier developed the theory that plants are able to accommodate themselves to a range of habitats, the extent of accommodation varying with different species. He contended that changes in the environment call forth adjustments in form which, in time, become fixed; but when a species is transplanted outside of its natural range of occurrence, it grows poorly or fails to survive, for there are limits to the adjustments it can make. The capacity for morphological and physiological change, nevertheless, is wide, and corresponds to the variation which we find within polymorphic species, and sometimes even to that within groups closely related but generally recognized as separate species. Thus, units generally treated by taxonomists as subspecies, varieties, or minor variations would, by Bonnier's theory, be merely morphological expressions of the same thing in different environments, more or less fixed in character, however, by reason of having been acted upon by their environments over long periods of time.

Confirmation of Bonnier's results has been claimed by Clements (23), who experimented along an altitudinal transect on Pikes Peak, Colorado, where stations were established at elevations ranging between 1820 m. and 3960 m. Another station is located at Santa Barbara, California. Examples of the numerous transformations reported by Clements (24) are *Erigeron salsuginosus* into *E. uniflorus*, the subalpine *Solidago Virgaurea multiradiata* into *S. missouriensis* of the plains, *Epilobium angustifolium* into *E. latifolium*, repeated conversions of *Phleum pratense* into *P. alpinum*, and the reciprocal conversion *Phleum alpinum* into *P. pratense*. Since the experimental evidence of Clements' investigations has not yet been published, a critical evaluation of his work cannot be made at the present time.

Following several years' association with Clements in the transplant experiments on Pikes Peak, Hall (44) started extensive experiments along similar lines in California in 1922. These he continued until his untimely death in 1932, after which they were carried on by his associates who have reported this work in a current publication (20). These investigations were conducted along a transect in California ranging from the coast near sea-level to the summit of the Sierra Nevada near timber line. Three transplant stations were used principally, one at Stanford University near the coast at 30 m. elevation, another near Mather, Tuolumne County, on the western slope of the Sierra Nevada at 1400 m., and a third in Slate Creek Valley, near Tioga Pass, Mono County, at 3050 m. at the crest of the range.

One hundred and eighty-two species were tried, but only about fifty proved to be suitable for transplanting experiments. Clone-members of plants dug in the wild were first established in a nursery and then transplanted to the different altitudes. Records in the form of herbarium specimens, measurements, and seasonal notes were taken methodically at suitable intervals for a number of years. Comparisons were made in two directions, (1) between clone-members grown simultaneously at different altitudes, and (2) between individuals of the same or closely related species brought from diverse native environments into the same garden. These data were supplemented by cytological studies on chromosome number and meiotic behavior, and, within some groups, by crossing experiments as well. The principal groups studied included

Potentilla glandulosa Lindl. and its relatives, *P. gracilis* Dougl. and related species, *P. Breweri* Wats., *P. Drummondii* Lehm., the genus *Zauschneria*, *Penstemon procerus* Dougl., the *Achillea millefolium* L. complex, and the *Artemisia vulgaris* L. complex.

These investigations do not substantiate the conclusions of Bonnier and Clements, that altitudinal races can be interconverted by transplanting. Not one of the hundreds of experiments of Hall and his associates gave any evidence of such a transformation. In some plants the clone-members were subjected to contrasting altitudes for as long as 16 years. The principal conclusions drawn from these studies are as follows:

1. Every plant retains its individuality regardless of the station or conditions to which it has been transplanted.
2. Every plant is subject to a certain amount of modification, depending upon the environments to which it is exposed.
3. The extent and nature of these modifications vary with different species, subspecies, races, and even individuals, *i.e.*, a plant's reactions to different environments are as much a part of its characteristics as any morphological feature. Moreover, the effects of a series of environments on a given plant are quite unpredictable.
4. These modifications are temporary, reversible, and quickly induced. Meristems giving rise to new tissues in the new habitats develop organs with the modified type of architecture.
5. Modifications often parallel differences that are hereditary. It is, therefore, impossible to distinguish between hereditary differences and modifications except by experiment.
6. Alpine races, as well as intermediate and intergrading forms, remain morphologically distinct from lowland forms of the same species when grown beside them, either at high or low elevations.
7. Modifications induced on lowland forms brought to high altitudes sometimes cause them superficially to resemble alpine types of the same species. However, a careful study of distinguishing structural details of the qualitative sort, such as habit, character of flowers and inflorescence, branching, arrangement of nodes, venation, toothing of leaves, arrangement of leaflets, and nature of pubescence, serves to identify each form and each individual.
8. Physiological characteristics, like differences in earliness and capacity to survive, remain equally distinctive in all environments. Alpine races develop more rapidly than lowland forms of the same

species during the course of a season. The former produce foliage, flowers, and fruit in quick succession, thereby completing their cycle within a short growing period. They continue their speedy seasonal development when brought to lower elevations with a long growing season, but lowland forms are generally too slow in maturing to succeed at high elevations.

9. Forms of some species from the mild climate of the California coast are able to survive the more severe winters at the Sierran mid-altitudes, while others cannot; all eventually fail at the alpine station. On the other hand, alpine types of the same species sometimes survive well both at middle and low altitudes. Plants native to intermediate altitudes usually reach full development at the coast, but as a rule do not survive long at the alpine station. In general, altitudinal forms thrive best at that station whose climate is most like that of their native environments.

After careful consideration of Bonnier's evidence, and in the light of their experiences with the California experiments, Clausen, Keck, and Hiesey (20) consider that two weaknesses underlie Bonnier's experiments: (1) failure to insure the continued purity of his cultures, and (2) an unsatisfactory method in studying and interpreting the results. The necessity of frequent careful weeding to prevent contamination of cultures is clear to anyone who has had to maintain pure garden cultures; this Bonnier did not do, for he wanted to subject his transplants to all the vicissitudes of a natural environment, including competition with other plants. In view of the complex hereditary variations within one and the same species, the unpredictable nature of modifications brought about by transplanting to different environments, and the impossibility of distinguishing between hereditary differences and modifications except by careful comparisons in one or more uniform environments, it is easy to understand how results from long-time experiments could be misinterpreted, especially in the absence of complete herbarium records.

Other investigators have studied the effect of transplanting to different climates on a less extensive scale. On the basis of field observations made by himself and others, MacDougal (59) favored the theory of the inheritance of acquired characters, but he placed less emphasis on this view in later years after experimenting with cultures transplanted into different environments (61). Mac-

Dougal attempted to study the capacity of different kinds of plants to adapt themselves and survive in various habitats by distributing similar lots of seeds, bulbs, tubers, rhizomes or shoots of 139 species to each of four stations. These included a garden at Carmel, California, in a mild, equable, coastal climate; a desert station near Tucson, Arizona; a xero-montane plot in the Santa Catalina Mountains, near Tucson, at 1600 m. elevation; and a montane station on Mt. Lemmon of the same range at 2500 m. having twice the rainfall of the xero-montane plot. The failure or survival of the different species was noted from time to time during a period of 10 to 14 years.

Different classes of plants were found to behave differently. Long-lived individuals, such as plants of *Vitis*, *Juglans*, and *Sanguisorba*, persisted in the contrasting environments over considerable periods of time; several were able to reproduce themselves in some of the stations by seeds. Members of such genera as *Fragaria*, *Potentilla*, *Scrophularia*, *Verbascum* and *Opuntia* were able to establish and extend themselves by seeds, offshoots, or rootstocks. Bulbous plants, including *Lilium*, *Brodiaea* and *Arisaema* usually died out after several years at stations to which they were unsuited, apparently because of the gradual depletion of food in their storage organs. Many plants from high altitudes in the Santa Catalina Mountains thrived better at Carmel, on the California coast, than in the desert near Tucson. From these results MacDougal drew conclusions concerning the migrational movements of plants, but dealt very little with morphological changes resulting from transplanting.

Cockayne (25) at first favored the idea that modifications may become gradually fixed, so that a shift to a new environment may require a number of years to overcome the after-effect of the first set of stimuli. This was based on fragmentary evidence from *Coprosma propinqua* A. Cunn. He dug a plant of low, prostrate habit from an exposed maritime habitat on Chatham Island, and after growing it in a pot for three years at Christchurch, transplanted it to a protected garden at the Canterbury College botanical garden. For four more years it remained prostrate, but during its eighth year it suddenly became erect. A similar observation was made on a plant of *Coprosma cuneata* Hook. The theory that modifications may become fixed was also entertained by his co-

worker, Allan (1), who studied a number of plant-habitat relations in New Zealand, but in a later paper, Cockayne and Allan (26) waived the notion of fixity, leaving the question an open one. The revised viewpoint was evidently reached after further experimentation with garden cultures, and critical observations in the field.

The California poppy, *Eschscholtzia californica* Cham., is a variable species in its natural range. Jepson (45), observing different stages of seasonal development and the behavior of transplants brought from the interior valleys of California to the coast at Berkeley, concluded that the diversity found in the field is due in part to morphological modification brought about by different habitats, in part to progressive seasonal changes, and finally to constant lines of variation. Only the more prominently differentiated of the latter kind of differences are considered by Jepson to justify taxonomic recognition as varieties.

An effect of salt spray in causing the sloping, wind-swept appearance of coastal shrubs through injury to young shoot tips has been observed by Wells and Shunk (86) on *Myrica cerifera* L. and other shrubs of the North Carolina coast. Salt solutions sprayed on experimental plants were found to cause injury similar to that observed on natural plants facing the sea.

A study of native habitat forms of *Atriplex semibaccata* Brown at San Pedro, California, ranging from the zone immediately along the ocean shore to points three miles inland is reported by Bullock (16) who attempted to relate differences in growth to external factors under observation, such as temperature, evaporation, soil moisture, and salinity.

Bouget (10), continuing investigations begun by Bonnier, studied the effect of transplanting lowland and subalpine forms of *Poa annua* L. to an alpine plot at the Pic du Midi at 2850 m., and noted changes in the fruiting habits of both. When he grew seedlings of the lowland annual fruiting form beside turfs of the rootstock-producing (but seedless) subalpine perennial at the alpine plot, above the natural range of both races, the lowland form remained an annual, and the subalpine a perennial, but the latter produced seeds like the lowland form. That the fruiting and growth habits of the *Poa annua* group are complicated by both environmental and hereditary factors is indicated in the study by Nannfeldt (68).

An experiment whereby parts of pure lines were exposed to different climates for ten generations in a ten-year period was reported by Christie and Gran (17). Seven strains of oats and two of barley were sown at six experiment stations in Norway ranging from $58^{\circ} 51'$ to $65^{\circ} 50'$ N. Lat. Some of these stations were on the humid coastal side of the country, others on the drier continental side. Reactional differences between the strains were observed at the stations during these years. The reactions listed include: time required for germination and maturation, and the length, width, and number of culms produced. The various strains were modified to some extent at all stations, but they tended to keep their relative positions in earliness, and in length and width of stems. The tallest plants were produced at the southern coastal and the northernmost station. The most rapid maturation took place at the most continental station in the south, and the slowest at the southernmost coastal station, where the plants were tallest. After ten years, when the strains were all brought back to a seventh central station for comparison, there was no effect of the differential influences they had been under for ten generations.

In an attempt to demonstrate the inheritance of acquired characters, Lesage (56, 57) has conducted a series of experiments with the annual species, *Lepidium sativum* L. He found that the offspring of plants grown in a greenhouse for two or more generations were more precocious than the offspring of plants which had always been grown outside. This difference in earliness persisted through as many as 17 generations. Lesage attributed the difference to the effect of higher temperatures in the greenhouse, and concludes that he has demonstrated a case of a hereditary increase in precocity through an environmental effect. He grew his cultures for many years at various stations, principally at Rennes, Marseilles, Algiers and Rothamsted. At each of these places, the differences in precocity were retained. Moreover, Lesage found that strains grown for three generations at Algiers, the most southern latitude, became more precocious than the same strains grown continuously at Rennes, a cooler more northern latitude. Like the greenhouse cultures which had become early, the offspring of the plants grown at Algiers persisted in their acquired precocity for at least 9 generations when brought back to Rennes. Lesage reported a similar effect on plants sown in the late, warmer part of the season, as compared with plants sown in early spring.

Less clear-cut results were obtained with a dwarf variety of garden peas grown under glass and in the open, but in a recent report (57) Lesage points to results that tend to confirm those obtained with *Lepidium*. The weak point in Lesage's experiments appears to lie in his failure to demonstrate that his original stocks were pure lines and remained so, and thus to prove that the differential treatments, *i.e.*, open air *vs.* greenhouse, northern *vs.* southern latitudes, and early *vs.* late sowing, caused the hereditary differences in precocity. To the reviewer it seems likely that these environments favored a differential selection of late and early hereditary strains among his original seeds.

Evans (31) reported a study involving the planting of clone-members of ten early to late races of timothy, *Phleum pratense* L., at ten stations located over a range of 28 degrees of latitude, from Gainesville, Florida, to Fort Vermilion, Alberta. Modifications in morphological characters and in relative earliness were reported. At Washington, D. C., at 38° 9' N., the ten varieties tested varied in the appearance of their first florets in 1935 between June 3 for the earliest variety and August 12 for the latest, whereas at Beaverlodge, Alberta, at 55° 2' N., the same plants started to flower on July 13 and July 21, respectively. The reduction in spread of flowering between the early and late races at stations at intermediate latitudes was progressive from south to north for the early varieties. The four latest began to bloom first at some station north of the most southern one, and from there the season for blooming progressed both toward the north and also toward the south. Only the very earliest variety thrived and flowered at the two southernmost stations at Gainesville and Belle Glade, Florida, whereas all prospered and matured at the most northern latitudes. These results were interpreted as being due to the differential effects of varying temperatures and day lengths at different latitudes on the heading, blooming, and maturing of early and late varieties. The work of Evans and his co-workers suggests a new trend in the investigation of climatic effects on plants in the light of advances made in plant physiology (32).

Marsden-Jones and Turrill (63), working in behalf of the British Ecological Society, set out to study the effect of transplanting a few selected species of British plants to different edaphic conditions. To this end they filled four large rectangular bins with

different soils, including a sand, a calcareous sand, a clay, and a chalky clay. In each bin they planted seedlings or divisions of selected species, including *Centaurea nemoralis* Jord., *Silene vulgaris* Garcke, *S. maritima* With., *Anthyllis vulneraria* L., *Plantago major* L., *Fragaria vesca* L., *Phleum pratense* L., and *P. nodosum* L. After ten years, they were unable to discover any mutations or long sustained edaphic effects. Various modifications were observed in the different soils, especially in *Plantago major*, but these were complex and unpredictable, leading the authors to conclude that every species is "a law unto itself." Marsden-Jones and Turrill emphasize the complex factors involved in such an experiment, a point that needs no elaboration to those familiar with soil-plant relationships.

Aquatic and amphibious plants have long been known to undergo spectacular structural changes when growing alternately as terrestrials and as aquatics. In his well-known paper, Massart (64) clarified a taxonomic problem in *Polygonum amphibium* L. by demonstrating that propagules of the same individual grown on moist land, dry sand dunes, and in water produced three morphologically very distinct plants. Massart pointed out that such amphibious species as *Veronica Anagallis* L., *Ranunculus aquatilis* L., and *Polygonum amphibium* L. may grow in a wide range of conditions, and are able to modify their structure according to circumstances, whereas more specialized plants such as *Chara* or *Nitella* are restricted by their hereditary protoplasmic structure to a more fixed pattern of development.

MacDougal (60) studied the growth of the amphibious plant *Neobeckia aquatica* Greene under a wide range of conditions over a period of 12 years. He grew this species on land and in aquaria at the New York Botanical Garden, in a greenhouse at the Desert Laboratory at Tucson, in the Santa Catalina Mountains, Arizona, at 2440 m. elevation, and at a cool, foggy, coastal station at Carmel, California. This plant has the capacity to thrive under a very wide range of conditions, producing very different kinds of stems and leaves in different environments, the most contrasting of which are those developed on land and in water. MacDougal studied leaf modifications in detail, and concluded that while environmental factors determine their structure, reactions to external agencies do not involve a direct physical adjustment, but a complex interaction between environment and the plant's protoplasm.

Glück (33) assembled a large amount of data describing the growth and life histories of aquatic and marsh plants. In his cultures at the University of Heidelberg, plants of the same species were grown on land, in different depths of water, and in reduced light for the purpose of studying their reactions, fruiting habits, and seasonal cycle of growth under different conditions. Glück's comprehensive work embodies many years of field and experimental study. From his monographs it is evident that the capacity of these plants to produce different kinds of leaves, stems, and variations in anatomical structure in different habitats is a characteristic as peculiar to each species as any morphological character.

Through cultural studies, Setchell (72) demonstrated the hereditary nature of the differences between two varieties of *Ruppia maritima* L.; one, var. *rostrata* Agardh., occurs in shallow pools connected with outer salt water at Richardson's Bay, California, while var. *longipes* Hagström grows in the Bay itself in the zone between the tide levels, where it is alternately exposed to air, shallow, and deep water.

The effect of different environments on mosses has been investigated by Davy de Virville (28) who grew ten to thirteen species in enclosed chambers under different conditions of light, temperature, humidity, and in different media such as on soil, under a covering of soil, and under water. The modifications induced were appreciable and sometimes quite striking. The extent of morphological and anatomical modification varied with the species, some changes affecting taxonomic characters. Thus, under certain conditions leaves of *Mnium undulatum* Neck. no longer fitted the descriptions of floras. *Polytrichum formosum* Hedw., considered to be the most complex and advanced species in evolutionary development of those investigated, became the most modified, while species of *Hypnum*, of simpler structure, changed relatively little. The xerophyte, *Racomitrium lanuginosum* Brid., which grows on dry rocks and in sunny places, thrived well in humid and even in aquatic habitats, becoming considerably modified. Strong light tended to inhibit growth in the species studied, except in *Racomitrium lanuginosum*, which grew well in high as well as in low light intensities.

Davy de Virville found analogies between the modifications which he produced in the laboratory and the variations found in nature. In discussing the application of his results to the taxonomy of

mosses, this author points out various key characters which he found to be modifiable, such as shape and size of leaves, although in general these changes were characteristic for each group, and each species as a whole retained its differentiating traits.

In his taxonomic studies in the hepatics, Buch (13, 14, 15) subjected selected species to culture tests to determine their reactions under different conditions of light, humidity, and temperature. The plants were grown in sash frames for a period of time under one set of conditions, after which one or more of the variables studied were changed. Species previously thought to be distinct, as for example *Scapania undulata* (L.) Dum. and *S. dentata* Dum., were found to be modifications of the same thing, while, on the other hand, new species were discovered through their reactions in culture. Thus, *Lophozia gracillima* Buch was found to differ from *L. longidens* Lindb., with which it was formerly associated, through its different pigment reactions in strong and weak light. The two are distinguishable also through differences in arrangement of leaves and size of cells.

Buch adopted a system of descriptive nomenclature (14) for naming modifications to distinguish them from hereditary differences. The categories *species* and *varietas* are reserved for differences that are inherited, while the term *modificatio* (abbreviated mod.) is applied to changes that are induced by the environment. Thus, *Calypogeia Meylanii* Buch mod. *laxifolia* has long stem segments, brought about by growth in reduced light, while mod. *densifolia* has short stem segments, produced in strong light. This system, applied to a list of typical changes brought about by ordinary environmental differences, is designed to depict something of the capacity of the various species to alter their morphology in various habitats. The term *modification*, early introduced by Nägeli, was popularized in botanical literature by Baur (4), who emphasized the distinction between modifications and hereditary differences. Other terms have been proposed to designate changes induced by the environment, including *accomodat* by Massart (64), *ecad* by Clements (22), *ecophene* by Turesson (75), *epharmonie* by Cockayne and Allan (26), and *phase* by Cockerell (27). These terms have various shades of meaning, depending upon the concepts of their authors respecting the significance of environmentally-induced modifications.

HEREDITARY VARIABILITY WITHIN SPECIES

The effects of natural environments on plants cannot be discussed adequately without introducing an important body of evidence accumulated by investigators who have grown living specimens of closely related plant forms originally from diverse environments in a uniform garden for the purpose of comparing them. The classical studies of Jordan (47, 48) on *Viola* and *Erophila* and of Nägeli (67) on *Hieracium* stimulated much discussion and investigation, particularly among geneticists and cytologists of a more recent era. Both Jordan and Nägeli proved the hereditary nature of numerous forms within groups considered to be species or species-complexes, and paved the way for a field of research that may still be considered to be in its beginnings.

In more recent years Turesson (75, 76, 77, 78, 79, 80) has advanced the work begun by his predecessors, and has greatly clarified our understanding of the relationships between plants and their environment. His work has led to new conceptions that are gaining increasing support from many workers. Turesson brought plants from many habitats and grew them in a uniform garden at Åkarp, in south Sweden. Twenty or more individuals were taken in each collection, thus including possible variants within a given population. By comparing many such collections in a standard environment and performing genetic tests on them, Turesson assembled a unique and illuminating mass of data. Species having a wide and continuous geographical distribution over a variety of climates were especially sought for study. While many of his materials were obtained from different places in Sweden, he also assembled plants from Norway, the Faeröes, England, Scotland, Denmark, Germany, Austria, Italy, Russia and Siberia. His extensive cultures permitted a statistical comparison of populations of the same species from contrasting habitats.

Turesson demonstrated several important principles relating to species composition. Among the most significant is his proof that the variations that occur within species are mostly hereditary. Thus, forms that grow along exposed sea-coasts with thick leaves and prostrate habit usually differ genetically as well as phenotypically from inland plants of the same species with thin leaves and erect habit. Alpine dwarfs with reduced inflorescences and dense cushions of basal leaves have a different hereditary constitution

from plants of low altitude and tall habit, spreading, bulky growth and slow seasonal development. Likewise, plants from the south are taller and later than forms of the same species further north because of inherited differences. Plants from certain regions may have other combinations of characters, as in populations in the Altai Mountains in South Siberia, where Turesson found races combining large size with earliness of flowering (80). In some species the number of hereditary races is very large, as for example in *Melandrium rubrum* (Weig.) Garcke, *Solidago Virgaurea* L., *Atriplex patulum* L., *Sedum maximum* (L.) Suter., *Armeria vulgaris* Willd., and *Hieracium umbellatum* L.

The great majority of the variations within species were found to be hereditary, each remaining distinct when grown side by side, although each had a certain capacity for modification, especially striking in plants from extremely exposed places such as windy sea-coasts or alpine habitats. Here, in a protected garden, they increased in stature the first year or two after transplanting, following which they usually remained at a constant height in succeeding years except for slight fluctuations due to seasonal differences. It was impossible, however, to predict without such experiments whether variations observed in the wild were hereditary or merely modificatory. Moreover, some natural populations consisting of mixtures of hereditary types which could not be distinguished in the wild were not discovered until they were cultivated in a uniform protected garden.

Turesson visualizes the climate as a selective agent which controls the kinds of forms that occur in a given habitat. Thus, mutations or other hereditary variations occurring in the population of a given species may give rise to plants better or less able to compete with other plants in the same environment. These are encouraged or eliminated, depending upon their suitability or unfitness for a particular habitat as compared to their competitors of the same or different species. Turesson stresses the heterogeneity of genotypes in populations of many species at any given habitat. If one transplants a random sample of a species, composed of twenty or more individuals growing in close proximity with each other in the wild, individual genetic differences become evident under cultivation in a uniform garden. These represent genic combinations which are all able to survive in one environment; in an unlike habitat, some would

be eliminated, while new combinations might arise which were unsuited to the first situation. The process of selection of hereditary forms by the environment, carried on through long periods of time, over a wide range of habitats in which a species may be distributed, results in the present close correlation that we observe between forms of a species and their surroundings.

Turesson (76) calls this field of study *genecology*, and has proposed a classification of plants based on genecologic concepts. His basic unit is the *ecotype*, a group which is recognizably distinct as a result of the selection of suitable hereditary forms by a particular habitat. These principles have found strong support in the work of various other investigators, including Hall and his associates, already reviewed, who demonstrated the genetic basis for ecotypes in *Potentilla glandulosa* and *Zauschneria*. An analysis of *Viola tricolor* by J. Clausen (18) revealed the occurrence of ecotypes, and later genetic investigations by the same author (19) proved their hereditary nature. Gregor and his co-workers (37, 40) have shown the existence of many hereditary forms, and a parallelism between modifications and hereditary variations in *Plantago maritima* L. Stapledon (74), in genetic investigations on races of *Dactylis glomerata* L. from different sources, has emphasized the numerous hereditary forms of the cocksfoot grass. Gregor and Sansome (41) have proven the existence of definite, genetically distinct habitat types in the grasses *Lolium perenne* L., *Dactylis glomerata* L., *Phleum pratense* L., and *P. alpinum* L. Gravis (35) has presented experimental data with races of *Plantago Coronopus* L. from the French and Belgian coast to which a similar interpretation may be given.

The hereditary nature of the differences between the prostrate maritime form of *Baccharis pilularis* DC. of central California (ssp. *typica*) and the erect, generally more inland form of greater geographical range (ssp. *consanguinea*) has been shown by Wolf (89) through cultural studies at the Rancho Santa Ana Botanical Garden. Seeds of *typica* gathered from wild plants were found to develop individuals as prostrate as the parents when grown in cultivation, in addition to other segregates of more ascending habit, none being as erect as *consanguinea*.

Lempert (55) has shown numerous hereditary races in *Dianthus* whose relationships cannot be determined by morphological studies

alone. Babcock and Stebbins (3) describe a situation in *Crepis runcinata* Torr. & Gray in which many forms showing regional differentiation occur. From a study of races of *Pinus ponderosa* Dougl. brought from nine climatic regions to a uniform environment at Priest River, Idaho, Kempff (49) concluded that strains native to the general region were better fitted than those of other regions. At the Institute of Forest Genetics of the U. S. Forest Service at Placerville, California, an extensive collection of altitudinal races of *Pinus ponderosa* and the related *P. Jeffreyi* A. Murr, is now being studied from the viewpoint of their suitability for different climates.

The idea of ecotypes fitted to given habitats has stimulated the breeding of agricultural crops suited to given regions. Russian botanists, largely through the leadership of Vavilov (82, 83, 84), have made great efforts in this direction and have utilized promising methods for the breeding of new horticultural varieties. The general procedure, summarized by Vavilov (84), Maleyev (62) and Sinskaya (73), includes the exploration and assembling of wild relatives from various habitats, the cytotaxonomic and genetic study of the materials, crossings with horticultural varieties, and selection of the progeny. Russian breeders have given special attention to the development of wheats and other cereals, potatoes, legumes, and fruits.

Gregor (39), on the basis of experience in the breeding of grasses at the Scottish Plant Breeding Station at Edinburgh, advocates the development of suitable "agroecotypes" at strategically located regional experiment stations. Bruman (12) likewise stresses the advantage of fitting agricultural crops to the regions they serve. Wheat (85), corn (46, 52), barley (65), timothy (38), and timber trees (42, 53, 54, 66) are among crops studied for which the interrelation between heredity and environmental requirements is receiving increasing attention, but developments in this direction can scarcely be said to have more than begun. It is significant that while horticulturists have more or less intuitively selected plants suitable for given climates and soils for hundreds of years, the analysis of the principle of regional differentiation in wild species, as determined from purely scientific studies, is needed to stimulate and guide conscious systematic selection of crop plants suited to given climates.

SUMMARY AND CONCLUSIONS

The experimental study of the effects of natural environments upon the characters of wild plants is beset with so many variables that it is not surprising that much confusion has existed on the subject. An understanding of the composition of the more common plant species is first essential. Botanical science is just now reaching a point where the hereditary complexity of the average species is becoming fully realized. The principle that plants are fitted by heredity to their natural environments, not only with respect to different species, but within the same species, emerges as one of the most significant facts from experiments directed towards the study of the effects of different natural environments on plants. With this point now becoming firmly established, specialists in various branches of botany can converge their efforts towards the further elucidation of the complex interrelations between plants and their environment.

Important current researches devoted to the study of the effects of certain environmental factors as, for example, plant hormones (87) and vitamins (6), length of photoperiods (32), effects of pre-treatments on seedling development (88), and agencies causing sex reversal (58), all have the common problem of dealing with the effects of environment when heredity is constant, or of variable heredity under a constant environment. When the factors of heredity and environment are confused, as has unfortunately been the case in studies on the effect of natural environments on natural populations, erroneous interpretations have resulted, for the interaction between heredity and environment imposes a complex of effects that cannot be separated.

Recent developments in the experimental alteration of chromosome number, the causing of chromosomal rearrangements, and the induction of mutations through temperature effects (71), aging of seed (69), radiation (34), drugs and growth hormones (5, 30, 36, 70), now make it clear that external factors may produce rearrangements or changes in the germ plasm. While there is no acceptable data at the present time to show that natural environments directly induce changes that become fixed in heredity, the possibility of externally induced hereditary changes through some action on the germ plasm must now be admitted. Developments in experimental cytology are so new that the feasibility of applying its techniques

toward synthesizing new forms of plants capable of maintaining themselves in the wild remains to be tested.

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MICROINCINERATION AND ASH ANALYSIS

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I. INTRODUCTION

The ashing of plant materials, including cut sections, on a microscopical scale is by no means a recent development. One of the earliest to report its value as a technic was Raspail (see 58) in 1833, who ashed epidermal layers of onion bulbs. In particular, siliceous skeletons of various plant families have been an object of study for nearly a century, having been investigated by von Mohl (33) in 1861. In 1914, Netolitzky (36) incinerated sectioned grains of prehistoric cereal remains as an aid to their identification. But the current cycle of development and application of the microincineration process owes its impetus primarily to the late Hans Molisch (34) on the botanical side and to A. Policard (43-46) in the field of animal histology. Since a plethora of excellent reviews on the animal and clinical phases of the subject already exists, only the botanical aspects of incineration data and technic will be considered here, with the exception of several strictly cytological investigations.

Extensive descriptions of representative technics from a clinical standpoint, which may also be of value to botanical workers, have been written from time to time by various workers in the field. Among the more critical and/or inclusive are articles by Policard (44, 46), Policard and Okkels (47, 48), Henckel (21), Schultz-Brauns (54), Herrmann (22, 23), Scott (58, 61), Gage (17), Baginski (2), Monsch (35), and Tschopp (67).

Photographs of the ash deposit from incinerated sections are often referred to as spodograms in the literature. Where sections are carbonized only, then the term anthracogram is applied to them.

II. TECHNICAL FACTORS

1. *Preparation of material*

Fixation. Since the usual aim of microincineration is to study the residual ash at the exact location it occupied in the living condition, the criterion of a perfectly prepared section is the maintenance of the *status quo* as regards both quantity and disposition

of the ash-yielding constituents. This restriction imposes severe limitations upon the various technics involved in the preparation of sections prior to incineration. In particular, the attempt to avoid both aqueous solutions, which would remove water-soluble compounds, and chemical fixatives containing metallic elements, which would contaminate the ash picture, has forced the employment of more difficult and less satisfactory methods of preparing materials.

The several technics frequently employed are: (1) the use of fresh material, (2) quick-freezing followed by dehydration in a vacuum, (3) the use of non-aqueous fluids such as alcohols; and, under proper conditions, (4) the traditional chemical fixatives. These will now be briefly reviewed.

Many objects or organisms can be placed directly on a slide and ashed without sectioning. Examples are leaves (7, 41, 72), blood corpuscles (29), *Spirogyra* (43), layers of plant epidermis (26, 67), fungus mycelium, *etc.* Still other objects may be sectioned fresh without any apparent chemical or physical alterations in structure. In this category come wood sections (37, 51, 69) and various other tissues which botanists are accustomed to cut in the fresh condition. In cases where more delicate tissues are being handled, the freezing-microtome can be brought into service. Relatively simple to operate, it permits sections at least as thin as 10 microns. Its use has been advocated by several animal histologists (21, 47, 48, 54, 67) as a prelude to microincineration, and it should be useful in botanical work as well. As will be pointed out in the succeeding paragraph, however, freezing methods are not always as applicable to plant as to animal tissue. The method has been adversely criticized by Scott (61).

The Altmann method of dehydrating tissue while in a frozen state in order to preserve it for microchemical analysis has recently been revived by Gersh (18) and further developed by Goodspeed and Uber (20) and Scott and Williams (62). The procedure consists of quickly freezing a tissue in a medium at the temperature of carbon dioxide snow or liquid air and dehydrating it *in vacuo* while maintaining a temperature below the eutectic point of the natural salts present in the material. Hoerr (24) recommends immersion in isopentane at -195°C for quicker freezing; alcohol or pentane may also be used. The theory of freezing living tissue without inducing other changes in it has been discussed by Goetz and Goetz

(19). Following dehydration, blocks of tissue are infiltrated with paraffin and sectioned in the usual manner, thus avoiding contact with water throughout the process. Although apparently quite satisfactory as a precursor for incineration in animal material, the character of the fixation in pollen mother cells of *Lilium* was not as good as expected by Goodspeed and Uber (20) from a cytological standpoint. As a histological technic for plants, it would probably serve very well.

The most widespread practice for fixation involves the use of a solution containing nine parts of absolute alcohol and one part of a strong neutral solution of formaldehyde, as long advocated by Policard (44) on the basis that most salts found in living tissue are insoluble in alcohol. Since water is introduced in the aqueous solution of formaldehyde, it does not appear essential to use absolute alcohol. Where sufficiently important, one could prepare a solution of formaldehyde in absolute alcohol, thus avoiding water completely. But since the mixture rapidly takes up water from the tissue, absolute alcohol would not seem to be required until dehydration was nearing completion. Incinerated preparations which were adjudged satisfactory (2, 27, 28) have been obtained with sections fixed in 95 per cent and as low as 80 per cent alcohol. Reed and Dufrenoy (52) used equal parts of 95 per cent alcohol and formalin for the fixation of orange leaves.

Just what percentage of the ash-yielding constituents are lost in fixing and other solutions is a controversial subject. Policard and Okkels (47, 48) report that 10–15 per cent of the potential ash of desiccated meat powder may be lost during their conventional fixation process in absolute alcohol and formalin. But Scott (61) contends this is not typical of regularly fixed preparations where tissues and cells are intact. Where the more aqueous solutions of alcohol are employed, the incinerated sections do appear to contain less ash (27, 68).

Fixatives containing metallic salts. It is generally assumed that fixatives containing chromium, mercury, osmic acid, *etc.*, are valueless for material to be incinerated (44, 47, 58). In opposition to this view, Gage (17) reports that thorough washing for 8–10 hours in running water and then in 67 per cent alcohol with frequent changes, will remove all but the slightest trace of chromium from tissue which has been fixed in a 3 per cent aqueous solution of

potassium dichromate. What such treatment would do to other ash-yielding constituents of the tissue is not discussed. Gage also adds that following the use of Helly's fluid the mercury can all be removed from tissue by the use of iodized 82 per cent alcohol. It is clear that such fluids give a superior type of fixation; that they may prove useful fixatives under carefully controlled conditions for particular objects to be incinerated also seems entirely possible.

The writer (68) of this review has had occasion to incinerate pollen mother cells of *Lilium longiflorum* and *Kniphofia* sp. which had been fixed in each of several of the conventional chemical fixatives containing inorganic salts. The results, hitherto unpublished, will now be described.

When mercury bichloride was used as a fixative, the cytological details were well preserved. Following a three hour period of incineration at 650° C, no positive test for mercury could be obtained with potassium iodide. Apparently all the mercury had evaporated, as would be expected from our knowledge of the behavior of inorganic mercury salts upon heating. The ash occurred in the form of small globules which were highly hygroscopic but not birefringent. Calcium was present as indicated by using picrolonic acid as a test reagent (9, 53). The localization of the ash in the sections was in accord with that obtained by the use of the freezing-drying method of fixation, the cytoplasm and chromosomes containing ash but the karyolymph being free from any deposit. This result substantiates the claims of Gage (17) referred to above and may justify the use of mercury fixation in cases where the nature of the results will permit an aqueous fixative.

With fixatives containing chromic acid and formaldehyde, such as Karpechenko's fluid, there was observed an abundant dense ash in the nuclei and a general deposit in the cytoplasm. Chromosomes were especially prominent when the slides were viewed with reflected light, indicating dense deposits. The colored ash obviously contained chromium salts. The presence of a deposit outside the boundary of the sections indicated that the ash had once been in a molten state. Likewise the appearance of the nuclear ash corroborated this impression since the entire nuclear area was uniformly covered with the characteristic colored deposit.

The substitution of pyridine for the formaldehyde in an ammonium dichromate solution gave sections with a different type of

ash picture. The heavy general deposits of dichromate-colored ash were now absent so that in a dark-field the background appeared black. The karyolymph was ash-free as in the technics where ash-yielding fixatives were avoided. The ash deposits had a globular character, cell walls appearing as a row of dots or minute hemispheres.

Trials were also made with various fluids containing one or more of the following: alcohol, formaldehyde, acetic acid, chloroform. Although the resultant fixation was of varying quality, the ash residues seemed to correspond in position with what would have been expected from the more approved methods. However, the quantity of ash appeared to be less in every case than sections of like thickness would yield if fixed by the freezing-drying method.

Barigozzi and Schreiber (6) made use of Bouin's fluid in fixing germ cells from the testicles of *Astacus saxatilis* preceding micro-incineration, and Barigozzi (3-5) employed Carnoy's and acetic acid solutions in fixing salivary gland preparations of *Chironomus* and *Drosophila*.

Imbedding and Sectioning. Paraffin has been employed almost exclusively as an imbedding medium, according to the conventional histological procedure. The ribbons obtained in sectioning readily enable alternate sections to be used for controls, if desired. The paraffin need not be removed prior to incineration unless, according to Gage (17), the fixing solution contains mercury.

In case the celloidin method is followed, it is necessary to remove the celloidin from the sections before ashing. Otherwise, the rapid combustion of the celloidin may disturb the ash from the entire section.

For cytological ash studies, sections must be cut thin in order to avoid confusion from the piling up of ash from overlying cell walls and other cell parts. The range of 1-5 microns has been found most desirable in such work.

Mounting sections. In order to avoid water completely, sections may be spread on the slide with the aid of absolute alcohol, paraffin oil, or by using needles only. With substances of low surface tension, like those just mentioned, sections do not spread well. Baginski (2) preferred to use 80 per cent alcohol which gave him satisfactory spreading for sections up to 10 microns thick and reportedly no loss of potential ash. Neither air nor spreading

fluid should be entrapped beneath the sections for it might give rise to distortion during the incineration process. Although there would seem to be a somewhat greater opportunity for loss of ash-yielding constituents from cut sections than from an intact block of tissue, yet unless water has been avoided in all previous steps of the preparation routine, there can be no strong objection to employing it for spreading, at least in as high a concentration as it occurred in the fixative.

Material for incineration often must be attached securely to the slide in order to counteract possible displacement owing to shrinkage. The problem is to find a satisfactory adhesive free from ash-yielding constituents. Albumin does not fulfill this requirement, although it has been used at times (29, 52). Schultz-Brauns (54) found that animal tissue sectioned on the freezing microtome adhered to the slide sufficiently well without any additional adhesive. But plant materials, such as leaves, woody tissues, *etc.* have a strong tendency to shrink during the ashing process. To prevent leaves from curling up, Werner (73) placed a cover slip over them. For sections of xylem, Uber and Goodspeed (69) employed de-ashed gelatin. Unfortunately, they did not find it absolutely ash-free, although it was usable under circumstances in which a trace of background ash was not confusing, as in cell wall studies. Perhaps an absolutely ash-free gelatin can still be prepared, or possibly a synthetic resin. Hardened linseed oil has been tried (51).

Slides. Sections may be mounted on ordinary glass microscope slides or cover slips, provided the incinerating temperature is not too high and the slides are supported on a plane surface. This support for the slides may be a translucent quartz plate ground smooth on one side (69), a strip of platinum (37-39), aluminum (72, 73), iron (50), or other satisfactory metal, or any refractory material with a smooth surface. By incinerating at temperatures of 500° C, Schultz-Brauns (54) was able to use nickel racks on which as many as 24 microscope slides could be placed in a vertical position in his furnace during ashing. Where one wishes the ash to fuse to the slide, a low melting point glass as in ordinary microscope slides has an advantage. Higher melting point glasses as Corex D and Pyrex are preferred by some workers (17) and are desirable if one wishes to ash at temperatures above 650° C, or if fusing of the ash to the slide is objectionable. Fused quartz slides have been used but are

not essential except in those cases where microchemical tests might be interfered with by elements found in glass. Crystal quartz slides may break readily during heating and are not recommended by Policard (44).

The use of opaque slides has been adopted by some workers for ashing leaves, where the ash does not adhere to the slide but may be lifted off in one piece. Ohara (37-39) used platinum; Werner (72, 73) aluminum; Molisch (34) and Satake (65) porcelain. Mica has also been tried (25, 50).

Where ash is to be studied by reflected light, opaque slides should prove satisfactory for the incineration of sectioned preparations. Aluminum and platinum are serviceable for this purpose, and other metals may do as well. Some types of ceramic surfaces might prove useful, perhaps by undergoing color reactions with specific elements in the ash. This has not been tried to the writer's knowledge.

2. Incineration process

Apparatus. The procedure for ashing microscopical sections varies widely. Liesegang (29) and Kisser (26) simply held the slides over a Bunsen burner, but the customary method is to use an electrically heated oven. A tubular furnace with an internal diameter large enough to accommodate a standard microscope slide has been frequently employed. Several models have been described (2, 17, 21, 54, 61, 69). Scott (61) designed a conveyor-type furnace with the tube having both ends open, thus permitting continuous operation. Translucent quartz provides a satisfactory and fairly inexpensive cylinder or tube. The quartz cylinder used by Uber and Goodspeed (69) had a diameter of 7.5 cm. and was 90 cm. long. It easily accommodated eight slides at one time.

Elaborate and expensive apparatus is not essential for obtaining valuable results, provided one guards against contamination and ash displacement. The use of an ordinary cone-type electric laboratory heater has proved satisfactory (30); likewise a muffle furnace (23). Usually provision must be made for a plate of some refractory material to prevent deformation of glass slides while in a plastic state.

Temperature and time. The temperature at which incineration takes place will depend to some extent on the tissue being ashed.

Complete ashing is also a function of time. It thus becomes difficult to give explicit directions. Some workers prefer the lowest practicable temperature in that it permits the use of ordinary glass slides and results in larger ash residues. Too high temperatures must be avoided for several reasons. The slides normally employed soften so that the ash readily fuses into the glass, thus making a quantitative ash estimate of little value. Of perhaps more importance, since the melting point of some inorganic salts is attained in the vicinity of 800°C , there is a decreasing amount of residue at the higher temperatures owing to volatilization. Other salts such as the carbonates and bicarbonates decompose to leave oxides of calcium, sodium, and magnesium. The phosphates present are probably retained as pyrophosphates.

Combinations of temperature and time which are advocated by several investigators will now be discussed. Earlier workers (44, 67) using animal tissues recommended that the temperature of the furnace be raised up to $500\text{--}600^{\circ}\text{C}$ in 15 minutes, then allowed to cool sufficiently for removal of the slides. To prevent shrinkage it was advised (48, 58) to raise the temperature very gradually for the first $100\text{--}200^{\circ}\text{C}$. Scott allows 25 minutes in the range of $200\text{--}650^{\circ}\text{C}$, specific recommendations for elapsed time at intermediate steps being also given (61).

Schultz-Brauns (54) found a temperature of 500°C entirely satisfactory for incinerating animal tissue and thereby secured a greater ash residue. He had a temperature indicator on his furnace and essential regulating rheostats. Baginski (2) recommended 400°C as a practical temperature for microincineration in order to retain a maximum of ash. At this temperature, the carbonates would not have decomposed. A stream of oxygen through the furnace was found to be desirable. A temperature of only $300\text{--}325^{\circ}\text{C}$ has been reported successful for the ashing of basswood sections (51).

That a temperature of only 450°C is satisfactory for ashing bulk plant samples in crucibles has been demonstrated by Stewart and Arthur (64). They used an oxygen supply, however, and over a period of eight hours, but obtained a white ash of excellent appearance. This ash was never fused. The lower incinerating temperature also resulted in a larger amount of ash, either from a reduction in evaporation or a lessened decomposition into volatile

reaction products as, for example, the carbonates to give carbon dioxide.

In case one is interested in only a carbonized section or anthracogram, a still lower temperature will suffice, 250° C being satisfactory. Such sections permit photography without staining. Carbonization by oxidizing agents can also be carried out on microscopical sections. An extensive discussion of carbonizing sections has been furnished by Kisser (26).

It is clear, of course, that the routines adopted in various laboratories are dependent to some extent on the nature of available apparatus, a determining factor frequently being the small furnaces without temperature regulating equipment. Where automatic regulators are used (69), quite constant conditions of temperature may be maintained indefinitely. For longer periods of incineration this is often essential, as in ashing at the lower incinerating temperatures.

Oxygen supply. Some workers (2, 51, 67) have felt that the circulation of oxygen through their furnaces hastened and improved the incineration of tissue sections; others advocated slower oxidation. To achieve the latter, several investigators (35, 54) have circulated nitrogen. Animal preparations ashed in nitrogen have been reported to form fewer tarry products (35) than if incinerated in air, and to contain more ash. The use of oxygen permitted lower incinerating temperatures, in the opinion of Baginski (2). Herrmann (22) saw no advantage in circulating either oxygen or nitrogen. Parker, Patzer, and Ritter (51) used oxygen plus a small percentage of ozone.

The fact that the period of time required for complete incineration depends on the type of tissue, its thickness, the temperature, and the availability of oxygen means, in practice, that one must test for complete incineration by observation of the residue. Usually this is done by microscopical examination under both bright and dark-field illumination in order to detect the presence of carbon particles (26).

Shrinkage. During incineration, practically all tissues, both plant and animal, undergo marked shrinkage unless steps are taken to prevent it. In woody sections, this tendency to shrink becomes most pronounced in the temperature range 300–350° C (71). There seems to be no known way to counteract shrinkage except by

exerting restraining forces. This can be accomplished more or less successfully by the use of strong adhesives for attaching sections to slides. Uber and Goodspeed (69, 71) found de-ashed gelatin satisfactory, except for a slight amount of residual ash. An absolutely ash-free gelatin is apparently unobtainable at present, at least commercially.

For animal tissues, the critical temperature for shrinkage has been reported by Policard (58) to be between 60 and 70° C. If this interval of temperature is passed through slowly, the sections are improved and shrinkage is pretty much confined to connective tissues. Policard and Ravault (49) preclude shrinkage during incineration by assuring a maximum contraction of the tissue before sectioning by bringing it to the boiling point in absolute alcohol. Schultz-Brauns (54) reports less shrinkage from material cut fresh with the freezing-microtome.

Preservation of Ash. Owing to the hygroscopic nature of the ash, it is frequently necessary to take special precautions to preserve it until observation and analysis can be made. The comparatively rugged ash skeletons of the genus *Equisetum* and of many leaves have usually been mounted in Canada balsam, aniline, or other liquid (34, 37, 72). Such treatment is unsatisfactory for the delicate and minute ash deposits from thin microscopic sections in that it disarrays the ash, prevents the subsequent addition of chemical reagents and interferes with optical characteristics essential for microscopical observation (61). It is often sufficient to attach a clean coverslip over the ash while still hot, making a moisture-proof seal with an appropriate wax. Scott (61) recommends a sealing preparation made up of one part each of paraffin, beeswax and resin. Gage (17) used pure beeswax; later applied a coating of shellac over the beeswax. Other workers (21, 47) advise the use of desiccators for storing ashed preparations. Still better is the suggestion of Schultz-Brauns (54) to place the slides in a drying oven maintained at 60–80° C.

3. Ash Observation and Analysis

Physical analysis. Information regarding the physical properties of the ash is obtained, for the most part, from observations with various types of microscopes. The bright-field microscope will enable the presence of carbon to be checked and also the general disposition of the ash, particularly the denser residues. For traces

of ash, the dark-field microscope has proved more valuable. Its use has been described at length by Gage (17) and others (58). Caution is essential with the dark-field, especially where the ash is dense or has been in a molten condition. In the latter case there may be semi-cylindrical or hemispherical residues which act as optical lenses and give false impressions of their actual shapes. Carbon particles in a dark-field exhibit a reddish color and may be mistaken for iron residues (28, 58). In every case it would seem advisable to check the appearance of the ash with both bright and dark-field, and if possible, also by reflected light. For example, a dense ash which would not transmit light with a dark-field condenser would appear exceedingly prominent by reflected light.

Identification of a microscopic ash deposit by its color is not feasible, in general. The color depends on the source of illumination, on the quantity and fineness of the ash (32), and on the method of observation, not to mention subjective factors introduced by the observer. Usually the ash is white or nearly so. Large quantities of certain salts would doubtless give a distinctive color, as chromium, for example. But salts likely to be present in appreciable quantities are not so conspicuous. Iron salts are reported to have a reddish color in the ash and to be readily identifiable (45, 48). Here, too, caution is necessary since carbon particles may also appear red in a dark-field (28, 58). Contamination from particles of iron off the microtome knife in sectioning is an obvious source of danger (58), against which one must guard.

The birefringence of ash has been noted by a number of investigators (58, 68), but it has not been sufficiently evaluated as a method of identification for ash constituents. Most crystals are double-refracting. That the birefringence of ashed sections is owing to silicon compounds has been indicated by Scott (58). This property of the ash might be useful in determining melting points with the polarizing microscope (see Vol. 1 of Chamot and Mason, 12) and thus leading to crystal identification.

The fact that ash has been observed to be highly hygroscopic in most cases does not aid materially in an analysis. Since the residues of many suspected elements following incineration would consist of deliquescent oxides or salts, specific clues are not furnished.

Quantitative estimation of ash. Several attempts have been made to secure a quantitative estimate of the amount of ash residue

from various types of tissue, both normal and pathological. Schultz-Brauns (54) used a photographic method depending on the density of the developed plate as a criterion of quantity. Quantitative photographic techniques for measuring radiation are quite involved in that they require the standardization of so many variables. The plate density of an image depends on the particular photographic emulsion, on the type of developer and the time and temperature of development, on the wave-length of the light, and further, the density is not constant for constant values of the product of light intensity and time of exposure. For relative estimates of ash quantity, it is necessary in addition to standardize the light source or keep it constant and maintain a uniform thickness of sectioned material for incineration. At best the results are only relative, not giving the ash quantity in terms of grams.

Scott (60, 61) and Scott and Williams (63) developed an elaborate photoelectric technic for measuring relative ash quantity which, though subject to several criticisms, is capable of greater accuracy than a photographic method. A dark-field condenser was employed. When using dark-field illumination, a thick deposit may well transmit less light than a thinner one. Reflected light would appear to be a somewhat better technic, but after a reflecting layer is once present, additional ash would not be quantitatively detectable. For this reason, sections only 4 microns thick were used.

In the present state of microincineration studies, it seems doubtful if such elaborate attempts to measure ash quantity are justifiable. In any case, one must standardize all of the numerous procedures involved, including time and temperature of incineration, fixation methods, thickness of sections, and others. Sections must necessarily be thin, and uniformity in thickness of thin sections is far from satisfactory from a quantitative standpoint. Then, too, various types of tissue incinerate at different rates. The fact that much of the ash may be bound into such organic constituents as nucleic acid or other phosphorus compounds decreases considerably the value of quantitative measurements on ash as a function of type of tissue.

Chemical analysis. Qualitative tests for various elements in a section involving many cells are readily carried out by precipitation or color reactions. Appropriate micro-tests may be found elsewhere (9, 12, 52, 74). Where tests for an individual cell only, or

perhaps a structure inside the nucleus, are the desiderata, the procedure is much more difficult. Several methods have been proposed for localizing the application of reagents to ash preparations. The addition of a micro-drop of a reagent by means of a micro-manipulator would seem a logical procedure, but it is not so simple in practice (2, 58). Spreading must be confined to a minute region, a problem not yet solved in terms of subcellular dimensions where ultra-clean slides offer the optimum opportunity for wetting, and almost instantaneous evaporation must be inhibited.

One attempt to overcome these difficulties has been the application of collodion or gelatin to the ash (31, 47, 74), allowing the reagent to diffuse through slowly. This method would seem to be suited for color and crystal reactions, provided the initial application of the collodion or gelatin did not displace the ash.

Where the incineration temperature is sufficiently high, the ash fuses to the slide. In this case, it is not readily displaced and a chemical reagent may be applied directly, perhaps by first laying a coverslip over the preparation and permitting the solution to flow under it. Herrmann (22, 23) has used this method to test for the presence of phosphates and magnesium; Reed and Dufrenoy (52) have also applied it in their histochemical study of mottled orange leaves.

The possibility of using gaseous reagents, though a most logical technic, has not been extensively explored. Hydrogen sulphide was tried by Tada (66) and Okkels (42) for the detection of lead, producing a black granulation in the ash. As pointed out by Scott (58), carbon deposits might interfere as well as other sulphides. Comparatively few reagents of interest are applicable in the gaseous state. The removal of specific ash components by gaseous reagents, whose reaction products are likewise volatile, may offer a solution to some special problem. For example, iron and nickel residues might be removed by carbon monoxide, giving the corresponding gaseous carbonyls of these metals. Chromium might be removed by the formation of gaseous chromyl chloride.

Tests for specific elements. Tests that have been used for individual elements or radicals will be summarized briefly in order to introduce the microincineration literature on the subject. The application of dilute sulphuric acid, which results in the formation of gypsum crystals in the presence of calcium, has been used by

several investigators (1, 2, 14, 27, 28). Picrolonic acid likewise forms characteristic crystals with calcium (9, 53, 68).

Magnesium has been detected by precipitation with sodium phosphate (2, 27, 28) and by the use of Hahn's reagent (22). The latter gives a blue color test similar to 8-hydroxyquinoline, $C_9H_6N \cdot OH$, which is also recommended as a sensitive test for magnesium (9, see page 40).

According to Policard, iron may be recognized by the red color of its oxide which occurs in the ash. This test has been criticized by Scott (58, 61) and others (28) as previously mentioned. Some investigators have tried the Berlin blue reaction (2).

An extensive search for a sensitive reagent for phosphates has been made by Herrmann (22, 23), who had most success with an acidified ammonium phosphomolybdate solution in the presence of strychnine nitrate. Others to use only the simple phosphomolybdate reagent were Ohara (37), Barigozzi (4), Caspersson and Schultz (11), and Czaja (14).

Tests for zinc have been reported by Reed and Dufrenoy (52); for lead by Tada (66) and Okkels (42); for copper and nickel by Prat (50), who applied rubeanic acid and dimethylglyoxime, respectively; for potassium by Baginski (2) and Kruszynski (27, 28), who used chloroplatinic acid; for sodium by Baginski (2); for silicon by Scott (58), who depended on its birefringent ash; for sulphates by Herrmann (23); for uranium by Policard and Okkels (47), who depended on its fluorescence when illuminated with ultra-violet light.

III. RESULTS

1. *Systematic Botany*

One of the earliest attempts to use spodograms for the identification of plant products was that of Netolitzky, who incinerated cereal grains from prehistoric deposits (36). The glume of millet, in particular, enabled a classification to be made whenever its characteristic siliceous epidermis was available for analysis. The carbonized remains were incinerated, and sections were made of the ash following the imbedding of the latter in paraffin or celloidin.

The employment of the microincineration technic more recently for purposes of systematic classification has been inspired by Molisch (34). He incinerated leaves and other parts of various

plants and obtained characteristic ash patterns due to calcium oxalate crystals, cystoliths, and siliceous skeletons of cell walls and hairs. Molisch did not resort to sections but used the entire leaves. He suggested the method be used in identifying plant products of commercial value such as drugs and foods, in order to detect adulteration.

Ohara (37) used the method at the suggestion of Molisch for the identification of industrially important woods. He was interested in the distribution of calcium, silicon and phosphates in woody tissues. He reported phosphate present in sections of *Tectona grandis* according to the reaction to ammonium phosphomolybdate, but since the silica which was found present also gives the same reaction as phosphate to this reagent, the result with respect to the latter remains in doubt. Ohara also worked on the identification of the woods found in Japanese paper (38) as well as on the barks of Japanese conifers (39), the latter from a systematic viewpoint.

Commercial barks, particularly those valuable in the tanning industry, were investigated by Czaplá (15). He made use of tangential and radial sections as well as cross-sections in arriving at characteristic ash pictures. All of his sections were so thick, 150–250 microns, that his observations were of little histological and histochemical importance, having been intended only as an aid in the identification of barks used for tanning. Of similar import is the work of Blabensteiner (8) on herbaceous drugstuffs, but his improved technic resulted in 20–30 micron sections. Identification, however, was based primarily on the topographical disposition of crystals. Likewise Ohara and Kondo (40) made studies on Japanese botanical pharmaceuticals (1929).

Of greater importance from the systematic standpoint is the work of Werner (72) on various species of Austrian meadow grasses. The Gramineae, in particular, have silicified epidermal cell walls which make these cells prominent in spodograms. The investigations of Ohki (41) on the classification of the Japanese bamboos constitute a still more ambitious undertaking which gave him highly satisfactory results. He was able to classify all species studied by means of ashed leaves only. Distinctions between species were in some cases very slight, however.

In applying spodograms for the classification of the Urticales, Satake (65) considered cystoliths the most important criteria with

calcium oxalate crystals next in importance. He found that spodograms alone furnish insufficient evidence for species differentiation, particularly for large genera, but that they may be useful up to the limit of genera for purposes of classification. Bigalke (7) confined her studies to the Urticaceae, but investigated 48 out of the 49 genera of this family. She found the spodograms to be distinctive as to species in the genera where her more complete studies were made.

2. Cytology

Nucleus and cytoplasm. Although the rather accurate correspondence of detail in incinerated sections to that of tissue prepared for observation with the best conventional technics has been the subject of remarks since the day of Raspail (see 17, 58), yet only recently have studies of cytological significance been reported. Even Policard (44) thought only in terms of histology. In 1930, Scott (55, 56, 57) and Funaoka and Ogata (16) published their observations on dividing cells. Scott found the mineral ash of the nucleus to be localized in the chromosomes in the secretory duct cells and acinar cells of the submaxillary gland of the guinea pig (55, 56). In a similar study made with epithelial cells from the skin of the tadpole and in germ cells from the testicle of the white rat, Scott (57) was able to observe incinerated chromosomes in the various stages of the mitotic cycle. He concluded that the major part of the fixed mineral matter is resident in the chromatin material at each stage of division, although an almost imperceptible deposit existed elsewhere in the nucleus. Scott interprets this evidence as indicating "that the bond between chromatin and the mineral elements is assuredly one of some strength." In the light of later studies by Uber and Goodspeed (70), Barigozzi (4), and Caspersson (10), it would appear that these deposits must be regarded as primarily phosphate residues from the nucleic acid of the chromosomes, although other elements may be present to some extent. The correspondence between ash pictures and ultra-violet photomicrographs of cells in division lend support to this latter view rather than to Scott's (59) conception of fixed minerals being responsible for both phenomena. It is clear from the data of Caspersson (10) that any absorption of radiation by calcium or other inorganic salts present would be hopelessly insufficient to account for the results obtained in ultra-violet photomicrography or spec-

trophotometry of chromosomes, but that the absorption by the chromosomes is primarily owing to their nucleic acid content. Since nucleic acid absorbs ultra-violet on account of its pyrimidine component rather than its phosphoric acid group, any topographical similarities between ultra-violet photomicrographs of intact chromosomes and their ash pictures is quite fortuitous. Recent data by Caspersson and Schultz (11) are in accord with the above interpretation. Their determinations of high ultra-violet extinction coefficients in onion root tips followed by their demonstration of phosphorus residues in incinerated control preparations lends further support to the original suggestion of Uber and Goodspeed (70) that nucleic acid is the principal ash-yielding substance in question. Just what other ash-yielding substances may be present in chromosomes is not yet known.

The incineration of pollen mother cells of *Vicia faba* in early division stages by Funaoka and Ogata (16) likewise revealed ash concentrated in the chromatic portions of the nucleus. Their low-power bright-field photomicrographs do not permit a detailed study of cytologic features, however, and their published descriptions are much too scanty. Alcohol was used as a fixative, and various solubility tests were conducted on the ash.

Following fixation by the freezing-drying technic already described, Uber and Goodspeed (70) incinerated 7-micron sections of anthers of *Lilium longiflorum* and of *Kniphofia* sp. A temperature of 550–600° C was usually maintained for a period of 2–3 hours. Their dark-field photomicrographs of meiotic stages reveal (1) an abundant ash residue from the chromosomes, (2) no ash in the karyolymph, (3) the presence of ash in the cytoplasm, and (4) in the cell walls. They pointed out that the ash-yielding substance of the chromosomes was probably nucleic acid and that the residue was therefore phosphate. A positive test for phosphorus was obtained.

Using 1–3 micron sections of nerve cells, Kruszynski (27) found depicted in the ash the topography of the nucleus, nucleoli, chromatin and protoplasm. No ash residues were found due to the nuclear sap or karyolymph, nor to the neurofibrils or "structureless" protoplasm. Conducting chemical tests with a micromanipulator, the nerve cell deposits were shown to contain calcium, potassium, magnesium, and iron. In the case of the magnesium test with

Na_2HPO_4 , ash from a large number of cells was essential for a positive reaction; for the other elements, an individual cell sufficed.

More recently Kruszynski (28) has reported further cytochemical investigations on the ash of epithelial, muscle, and nerve cells. Incinerating at 400°C or 500°C for 30–45 minutes, he found more ash at the lower temperature in sections which had been fixed in 95 per cent alcohol-formalin and cut at 2–5 microns. The sections were ashed on cover slips, which were later inverted for purposes of observation so that oil immersion objectives and a Chambers micromanipulator could be employed. Applying reagents in microdroplets with the manipulator, he secured tests for calcium (gypsum crystals) in nuclear walls but was unable to obtain crystals from nuclear ash alone. Kruszynski observed no positive test for iron and was highly skeptical of the results of others, who determined its presence on the basis of a red color in the ash. In his opinion, carbon particles were probably responsible for the color.

A similar study of cells from incinerated ovaries was made by Baginski (1), who observed ash from chromosomes during division stages. He likewise conducted tests for several elements, namely Ca, Si, Fe, and Na, but not on ash exclusively from chromosomes. He reported calcium in nuclear ash.

It has doubtless occurred to many workers to study the salivary gland chromosomes of *Drosophila* by microincineration, but the difficulties in the way of obtaining really significant results seem to have been too formidable. Recently, however, several papers have appeared by Barigozzi (3–6), who has incinerated the salivary gland chromosomes of *Chironomus* and *Drosophila*. Although unable at first to distinguish the bands in the ash picture, Barigozzi (4) reported that various regions of the chromosomes differed markedly in their ash content. Phosphates present were interpreted as residues of nucleic acid or of other phosphorus-containing substances. Previous to incineration, the salivary glands were fixed in Carnoy's solution, imbedded in paraffin, and sectioned at 5 microns. Ashing was done at 400 – 500°C , and observations were made with a Zeiss cardioid dark-field condenser.

In later studies (3, 5), Barigozzi reported that the chromatic transverse bands of the salivary gland chromosomes contained ash while the clear regions between these transverse bands did not. There may be objection to these results of Barigozzi's in that he

made use of aqueous fixatives including Carnoy's, Bouin's and acetic acid solutions, which might have removed certain ash-yielding constituents.

Cell walls. That ash is localized in cell walls appears evident from the photographs of incinerated epidermis of *Tradescantia* leaves by Tschopp (67). Numerous observers have noted similar ash pictures in various plant tissues. But whether ash is to be found in all layers of a cell wall and in what relative proportion it occurs in each part, has not been completely elucidated.

In a histochemical investigation on the rapidity of incineration of various cell structures in *Spirogyra*, Policard (43) found cell walls to ash more slowly than cytoplasm but more rapidly than chloroplast threads. The walls likewise were intermediate in ash content, the chloroplasts containing more, and the cytoplasm and cell sap less ash. He stated that the chloroplast ash was probably magnesium for the most part.

Czaja (14, see pages 564-565 and 588) has incinerated sections of turnip tissue in order to study the ash from the walls. If pectic substances were first removed, then he found no calcium or phosphorus in the ash. However, he concluded from his observations that calcium must also be present in the walls, elsewhere than in pectic material, as an insoluble organic compound.

A study of the topographical distribution of ash in wood cell walls has been made by Uber and Goodspeed (69, 71). In the xylem of *Taxodium* sp., it was found that the amount of gelatin adhesive required to maintain the sections *in situ* during incineration was so great that the gelatin was responsible for an appreciable, if not the total, ash deposit in the secondary walls. With less gelatin, the secondary walls shrank back against the primary walls, again making the question as to whether the secondary walls contained the ash-yielding substances indeterminate.

In the thick-walled pith cells of *Sequoia sempervirens*, however, diffuse ash residues were definitely localized in the secondary wall layers. This finding made it seem very probable that some ash residue would be obtained from secondary cell walls of wood specimens generally.

Parker, Patzer, and Ritter (51) conducted a similar study on sections of basswood. To inhibit shrinkage, they mounted their sections in a drop of fluid made of equal parts of ash-free linseed

oil and turpentine, in which the sections were subsequently hardened at room temperature. However, they reported that the secondary walls did shrink back toward the middle lamella upon incineration. Their ashing was carried out at 300–325° C in a current of oxygen plus a small quantity of ozone. They concluded that ash occurred in the “middle lamella.”

3. Plant Pathology

In investigating the effect of zinc and iron salts, applied to the soil or as a leaf spray, on the cell structure of mottled orange leaves, Reed and Dufrenoy (52) studied the distribution of these elements in the leaf tissue. Following fixation in equal parts of 95 per cent alcohol and formalin, imbedding in paraffin, and sectioning at 6–8 microns, the paraffin ribbons were attached to microscope slides with albumen. For ashing, the temperature was gradually raised to 500° C within a three-hour period, then maintained 2–3 hours longer between 500–600° C. Tests were made on the ash with sodium nitroprusside, which reacts with zinc to form the corresponding, but slightly soluble, zinc salt, $\text{Zn} \cdot \text{NO} \cdot \text{Fe}(\text{CN})_5 \cdot \text{H}_2\text{O}$ (see Chamot and Mason (12) vol. 2). Manganese nitroprusside crystals are indistinguishable from those of zinc and may have been present. The presence of zinc was found confined almost exclusively to the palisade cells of the leaves or to the periphery of the palisade cells. Meristematic tissues of buds also contained conspicuous accumulations of zinc as revealed by the formation of an abundance of nitroprusside crystals. The distribution of calcium, which occurs in the form of the oxalate in citrus leaves, has also been commented on by Reed and Dufrenoy, but their microincineration evidence adds little to what is already known from other methods of observation.

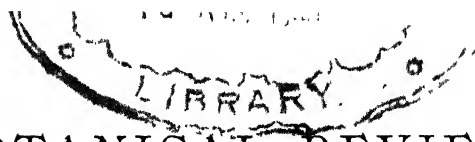
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THE GENOTYPIC BASIS OF SEX-EXPRESSION IN ANGIOSPERMS

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A consideration of this question is necessarily, at the present time, limited almost exclusively to the sporophytic ("asexual") generation. The gametophytic generation consists of sharply distinguished female and male individuals; but the distinction between these seems to be (save for the one or two exceptions noted below) irreversibly determined with the production by the sporophyte of pistils or of stamens. In the one case the line leads through ovary, nucellus, macrospore mother cell, and macrospore to macrogametophyte and egg; in the other, through anther, microspore mother cell, and microspore to microgametophyte and male gametes.

That the distinction between macro- and microgametophyte is not genotypically based appears evident from the fact that in angiosperms having recognizable sex chromosomes both microspores with the X and those with the Y chromosome develop into microgametophytes. It is reasonable to assume, though not yet demonstrated, that production from a micro- or macrospore of the corresponding gametophyte is determined by conditions outside the spore. The microspore germinates while lying free within the pollen sac; or, given an appropriate osmotic environment, without the sac. The germinating macrospore, on the contrary, is closely surrounded by layers of cells that may influence it in numerous ways, certainly including pressures which vary in different axes.

The most direct evidence at hand as to the alternate potentialities latent in either a macro- or a microspore is that supplied by the occasional development of a normally haploid microspore in certain varieties of *Hyacinthus orientalis* into an 8-nucleate, 7-celled structure closely resembling a macrogametophyte (embryo sac). Such embryo-sac-like structures, first observed by Němec (1898), have been more fully studied by De Mol (1922-1937), Stow (1930, 1933) and

Naithani (1937). De Mol and Stow agree that such development of a microspore in the female direction is induced by subjection to relatively high temperatures before or at the time of meiosis. De Mol holds that it is induced also by exposure to low temperatures (below 4.4° C.). However, the conditions under which the phenomenon in question was observed have been so various, as well as being complicated by the time of harvesting and by the methods of after-harvesting treatment followed by hyacinth-growers, that the relations of environment to the unusual development are still far from clear. Stow suggests also that a hormone supplied by adjacent dying or dead pollen grains may be of influence. The essentially female nature of these multicellular grains is supported in a measure by Stow's observations that they manifest a somewhat greater reducing power than do typical pollen grains, and that they seem in some cases to display a chemotactic attraction for pollen tubes. In one instance a tube penetrated the embryo-sac-like structure and within the cytoplasm of the latter discharged a male gamete nucleus.

Perhaps representing the converse possibility is a condition found by Heilborn (1931) in *Siparuna Eggersii*. Here a macrospore develops into a long tube-like structure which penetrates the chalazal region of the nucellus and apparently never produces an embryo sac.

The angiosperm sporophyte whose sex-expression is here to be considered is a diploid generation. In this respect it resembles the only generation of a metazoön. On the other hand, angiosperms differ from bryophytes (the other plant division in which sex-determination has been largely studied) in that in the latter the predominant generation which alone displays sexual differentiation is the haploid gametophyte.

Sex-expression in the angiosperm sporophyte consists primarily in the production of pistils (female), each formed by one or more carpels, and of stamens (male), which enclose the respective types of sporangia, spores, and, after spore-germination, the corresponding gametophytes. A single flower may be bisexual, including both functional pistil or pistils and functional stamens. Or pistils and stamens may be borne in separate (unisexual) flowers. Pistillate flowers (hereafter referred to as *female*) frequently, but not always, possess rudimentary stamens; rudimentary pistils often appear in staminate (*male*) flowers. Moreover, in structurally bisexual flow-

ers the organs of one sex or the other are sometimes imperfectly developed. If the imperfection reaches a point at which the organs in question are functionless, the flower is essentially unisexual. It follows that all degrees of intermediacy are to be found between strictly unisexual flowers with no trace of organs of the missing sex and flowers that are fully bisexual.

Another type of intergradation consists in the occurrence of organs which in varying degrees combine the structure of a pistil (or carpel) with that of a stamen. Wehrli (1892) cites an extensive list of observations of phenomena of this nature. Detailed descriptions of intersexual floral structures are given by Rainio for *Salix* (1927), *Papaver* (1929), and *Geranium* (1937).

In case flowers are unisexual, female and male flowers may differ in time of appearance, in position and grouping, or in the size and form of accessory organs (especially perianth leaves or sepals and petals). If flowers of the two types are borne on distinct plants, these female and male plants sometimes differ in habit, particularly in the form and profusion of branches. The divergent characters mentioned, apart from those distinguishing pistils and stamens, are often termed "secondary." Characters in this category described for various species are listed by Steckhan (1937). How closely they are comparable with characters similarly designated in animals is an open question.

It follows from what has been said that, omitting intergradations, flowers of 3 types may be present: bisexual, female, and male. If, as in the majority of angiosperms, the flowers are regularly bisexual, the species is hermaphroditic. If female and male flowers are characteristically present and are borne on separate plants, the species is dioecious. Between these extremes all possible conditions occur involving the appearance of flowers of 2 or 3 types on a single plant or their distribution among different plants of the same species. Some of these intermediate conditions are monoeicism, andro-, gyno-, and trimonoecism, andro- and gynodioecism. The distribution of the varied floral arrangements among families and genera of angiosperms has been summarized by C. and H. Yampolsky (1922).

Noteworthy is the frequent lack of constancy within a species as to floral types and their distribution. To class a species as andromonoecious, for example, may be to designate its frequent or usual

condition; but deviations from the described condition are reasonably certain to appear if large numbers of individual plants are examined. According to Schulz (1888), *Silene vulgaris* (*S. latifolia* (Mill.) Britten & Rendle) occurs in 5 forms: hermaphroditic, female, male, gynomonoecious, and andromonoecious. Correns (1908) distinguished 30 categories among plants of *Plantago lanceolata* (classed as gynodioecious), ranging from strictly hermaphroditic to female. In the majority of dioecious species (including, among others, species of *Urtica*, *Humulus*, *Cannabis*, *Spinacia*, and *Mercurialis*), male plants produce with varying frequency female or bisexual flowers; female plants of some species produce male or bisexual flowers. Indeed, as in *Mercurialis annua* (Yampolsky, 1930), strains of dioecious species occur which bear so large a proportion of flowers of both sexes as to be termed monoecious (better, perhaps, in *Mercurialis* trimonoecious). In a few species the sexes are more sharply separated. Of these, the most studied are *Lychnis dioica* and *L. alba* (known to Continental writers as *Melandrium rubrum* and *M. album*), and *Bryonia dioica*. Even here exceptions occur. For *Bryonia dioica* there is a report by Hy (1882) of the appearance of hermaphroditic flowers on an otherwise female plant. The development of stamens in originally female flowers of *Lychnis*, in consequence of infection by the anther smut, is well known (see, e.g., Strasburger, 1900). Occasional appearances of uninfected hermaphroditic individuals of *Lychnis* will be mentioned later.

Factors basic to sex-expression, like genetic factors in general, endow the plant possessing them with a certain range of possibilities of development. Which of the hereditary possibilities come to development, and to what extent, is determined by the reaction of the plant with its environment. These statements are almost axiomatic. But failure to recognize such simple facts has led to much bootless controversy.

A full explanation of the occurrence of sexual characters, as of characters of any other class, involves the correlation of two distinct lines of study directed toward the determination (1) of the behavior of individuals genetically alike under conditions as diverse as possible; (2) of the behavior under uniform conditions of genetically different but sexually compatible individuals and of their progeny from controlled self- and cross-matings. Investigations of the former type will determine the range of possibilities

inherent in a given genotypic constitution, and the influence of environment upon the expression of those possibilities. The literature in this field has been recently reviewed by Loehwing (1938). The second line of investigation mentioned aims at an analysis of the genotypic make-up of each species, variety, and strain. Work dealing with this type of study is the subject of the present review.

✓ EXPERIMENTAL STUDIES OF DIOECIOUS SPECIES

It is generally (not quite universally) agreed that hermaphroditism is the primitive floral condition in angiosperms. This conception being accepted, it follows that in various lines of descent many mutations have occurred which affected sex-expression; some of these, becoming fixed, have resulted in the establishment in particular species of monoecism, gynodioecism, and other intermediate conditions; the culmination of the series, in some lines, being dioecism. From a phylogenetic standpoint, then, the study of genetics of sex-inheritance might logically begin with hermaphroditic forms. The historical procedure has been the reverse; most of the earlier experimental work and indeed much that is more recent, has dealt with dioecious species. Experimentation with dioecious as well as with non-dioecious species has followed three main lines:

(1) Intraspecific mating. In dioecious forms this is expected to lead, and usually does lead, to the production of ♀ and ♂ offspring. In strictly hermaphroditic species it results in the production of hermaphroditic offspring. But in case the species (*e.g.*, a gynodioecious one) includes plants of two or more different types, matings between plants of these types may give significant results.

(2) Interspecific mating. Experiments of this nature are limited by the failure of many species to intercross, as well as by the frequent sterility of such interspecific hybrids as can be obtained. Fortunately, neither interspecific incompatibility nor hybrid sterility is universal.

(3) Matings involving typical and aberrant (mutant) forms of the same species. This has been the most fruitful line of investigation. In considering the results it must be remembered that the particular mutation concerned may have paralleled phylogeny—that is, it may represent progress in the general direction from hermaphroditism toward dioecism—or it may tend in the opposite direction.

In a hermaphroditic species almost any possible mutation affect-

ing sex-expression—excluding such as may involve change in form or number of ♀ or ♂ structures—must tend toward a diminution of female or of male-fertility or of both. Conversely, in a dioecious species almost any mutation affecting sex-expression, with exceptions of the nature just noted, must tend in the general direction of hermaphroditism—i.e., phylogenetically backward.

Following the historical course, studies of the genetics of dioecious species will be mentioned first, beginning with those whose dioecism is most strict; next, species will be considered in which sexual separation is less sharp, designated by Darwin (1877) "subdioecious." Most of the fragmentary and of necessity inadequately interpreted results obtained before 1900 will not be mentioned. References to this earlier work appear in later papers herein cited.

Bryonia. Prominent among the attempts, after the rediscovery of Mendel's papers, to elucidate the method of inheritance of sex are those of Correns (1907, 1928). (Reference is made here and elsewhere chiefly to Correns' summary discussions, which cite his long list of shorter papers.)

Matings of ♀♀ × ♂♂ of *B. dioica* gave a progeny including, as expected, approximately equal numbers of the two sexes. Further matings made by Correns, the maternal parent being first named, were:

B. dioica, dioecious × *B. alba*, monoecious. Offspring were all ♀, except that the first, usually abortive, flowers on some plants were ♂.

B. alba × *B. dioica*. About half the offspring were ♀, half ♂. Some ♀♀ produced at first a few abortive ♂ flowers.

These experiments were repeated, with similar results, by Bateson (1909).

Jones and Raynor (1915) crossed a race of *B. dioica* whose ♀ flowers were predominantly 2-carpellate with one having chiefly 3-carpellate flowers. In both F_1 and F_2 generations the proportions of 2-carpellate and 3-carpellate flowers varied greatly from plant to plant. It was concluded that the parent races differ in at least two genes, the 2-carpellate race being homozygous for both dominant alleles.

Lychmis. Correns' experiments with members of this genus (under the name *Melandrium*) paralleled, so far as successful matings were possible, those with *Bryonia*. Matings of ♀♀ × ♂♂ of

L. alba, previously made by Strasburger (1900), were repeated by Correns. Strasburger, as well as Shull (1911), mated ♀♀ and ♂♂ of *L. dioica*. The offspring in each case included approximately equal numbers of both sexes, although in general the ♀♀ were somewhat more numerous. This tendency toward female preponderance, common though not universal in dioecious species, will be referred to later. Mention may be made also of Béguinot's (1916) cross of *L. divaricata* × *alba* (both dioecious). Twenty offspring resulted, all ♀. One of these, back-crossed to *L. alba* ♂, gave a progeny of 57 ♀♀, 23 ♂♂.

Correns mated ♀♀ of *L. alba*, and of a hybrid (*L. alba* × *dioica*), both dioecious, with ♂♂ of the related *Silene viscosa*, hermaphroditic tending to gynomonocism. The offspring were all ♀♀, but with staminal rudiments somewhat more developed than in the mother plants.

From his results with *Bryonia* and *Lychnis* Correns concluded that (1) dioecism is almost completely dominant over monocism; (2) the eggs of the dioecious species studied carry a ♀ tendency, whereas half the ♂ gametes carry a ♂, half a ♀ tendency. It follows that the ♀ plant is homozygous and homogametic in terms of sex-tendencies; the ♂ is heterozygous and heterogametic, the segregation of sex tendencies occurring in meiosis. Bateson (1909), on the contrary, assumed heterozygosis in the ♀, homozygosis in the ♂. His explanation involved subsidiary hypotheses and has had little acceptance.

As a genetic basis for sex-expression, Correns (1928) assumed that all angiosperms possess potencies for femaleness, carried by a gene or gene-complex *G*, and for maleness, carried by a gene or gene-complex *A*. Another gene or gene-complex *Z* influences for each species the time and order of appearance of ♀ and ♂ organs. The sporophyte of a hermaphroditic or monocious species, then, possesses the genetic constitution *AAGZZZ*. A dioecious species has in addition genes ("realizers") tending respectively toward femaleness (γ) and toward maleness (α). He recognized the possibility that the γ and α genes may be negative rather than positive in their effects, each tending to inhibit the expression of the opposite sexual potency. The ♀ sporophyte in *Bryonia* or *Lychnis*, therefore, has the genetic formula *AAGZZZ* $\gamma\gamma$; the ♂, *AAGZZZ* $\alpha\alpha$. These formulae are presumably of general applica-

tion, although dioecious species may exist in which the ♀ is heterozygous ($\alpha\gamma$), the ♂ homozygous ($\alpha\alpha$).

While this hypothesis has been much used by Correns and others to explain the basic features of sex-expression, it has become evident that a more elaborate genic system must be evolved to account for deviations from the apparently simple condition of a sharp separation of the sexes. An illustration is the strain of *Lychnis* observed by Correns (1928) in which both petals and stamens were replaced by carpels. Another is the occurrence of thelygeny (thelytoky) in *Lychnis* and in some other dioecious forms. When a thelygenous ♂ is the pollen parent, the offspring are chiefly or exclusively ♀. The ♂♂, if any, among the progeny are in turn thelygenous; the ♀ offspring behave genetically as do other ♀♀ of the species. Thelygeny has appeared, too, in certain species and varieties of *Silene* (Correns, 1928; Newton, 1931). Correns explains the condition tentatively by a lethal or sublethal gene borne by the Y chromosome. The converse condition (arrhenogeny) was found by Shull (1914) in *Lychnis alba*. The offspring of matings involving certain ♂♂ of a narrow-leaved race were almost all ♂. Other occurrences of arrhenogeny will be cited on later pages.

Another complication is supplied by the "gynandromorph," also described by Correns, which was ♂ below, ♀ in its upper portion. The ♂ flowers were found by Bělář (1925) to possess the typical ♂ (XY) chromosome complement. The ♀ flowers pollinated from ♂ flowers of the same plant gave rise to ♀ offspring, 28% of which bore sterile flowers with reduced pistils. The ♀ flowers pollinated from ♂ plants produced ♀♀ and smaller numbers of ♂♂. Female plants pollinated from the gynandromorph produced ♀♀; in one family about one fourth of these were sterile.

Of a different order are the hermaphrodites, with more or less well-developed bisexual flowers, which have several times been reported in *Lychnis dioica* and *L. alba*. Shull (1910, 1911), who includes both species under the Linnaean name *L. dioica*, found several ♂♂ in cultures derived from typical dioecious ancestors. Certain of these ("genetic hermaphrodites"), or their hermaphroditic offspring, selfed, produced progenies composed of ♀♀ and ♂♂ (the ♀♀ somewhat in excess), and very rare ♂♂. Hermaphrodites as pollen parents, mated with ♀♀, gave a progeny of ♀♀, ♂♂, and rare ♂♂. A ♀ × a ♂ produced 29 ♀♀, 12 ♂♂, 2 ♂♂. Female offspring of

♀♀ mated with ♂♂ gave, like typical ♀♀, a progeny of ♀♀ and somewhat fewer ♂♂. Four "somatic hermaphrodites" (not self-fertile) mated with ♀♀ gave progenies consisting of ♀♀ and ♂♂. Since the genetic behavior of both classes of ♀♀ shows that they are heterozygous for sex-tendencies, and since, selfed or crossed, they behave as do normal ♂♂, save that in the "genetic" class ♀♀ replace ♂♂ among the offspring, Shull concluded that ♀♀ of both classes are modified ♂♂. He postulated for the genetic ♀♀ a mutation either of a ♂ or of a ♀ sex gene.

G. and P. Hertwig (1922) studied two hermaphroditic plants of *L. dioica* and their offspring. In some matings ♀♀ and ♂♂ of *L. alba* were used, but with no different results from those obtained with *L. dioica* plants. Two small families from matings of ♀♀ × ♂♂ included 14 ♀♀, 14 ♂♂, 14 ♀♀ (cf. Shull's corresponding figures of 29, 12, 2). Matings of ♀♀ × ♀♀ gave results similar to Shull's when his somatic ♀♀ were the pollen parents—nearly equal numbers of ♀♀ and ♂♂ plus, in this case, 3 ♀♀. The most marked divergence from Shull's results appeared in matings of ♀ offspring of ♀♀ with typical ♂♂; the offspring were 450 ♀♀, 330 ♂♂, 93 ♀♀; in Shull's case, 471 ♀♀, 305 ♂♂, and only 4 ♀♀. The Hertwigs explain the occasional ♂ offspring of selfed ♀♀ as genotypically hermaphroditic; growing slowly, they produced few flowers, and these ♂. Like Shull, they considered their ♀♀ genetic ♂♂ in which a gene affecting sex-expression had undergone mutation. Their formulated explanation is based upon Goldschmidt's theory of differences in the valency of the respective genes for femaleness and maleness. It assumes the occurrence of ♀♀ of 3, ♂♂ of 2, and ♀♀ of 2 genotypically different classes.

Winge's (1931b) results with hermaphrodites descended from crosses of *L. dioica* × *L. alba* are generally similar. He assumes, however, numerous genes tending, respectively, toward maleness and femaleness, borne on autosomes as well as on the X and Y chromosomes. Hermaphrodites result from a disturbed balance between these conflicting genes. One autosomal gene is recognized, tending strongly toward maleness, hence inhibiting hermaphroditism. A lethal recessive gene in the X chromosome when homozygous prevents the appearance of ♀♀.

The hermaphroditic condition in these exceptional plants, as pointed out by Correns (1928) and others, is different genetically

from the hermaphroditism regularly characteristic of the majority of angiosperms. "Secondary" hermaphrodites, such as those of *Lychnis*, have demonstrably arisen by backward mutation.

Still more complicated does the genetic situation appear in view of studies made upon less strictly dioecious species.

Salix. In 25 crosses made by Heribert Nilsson (1918), each involving two, three or more species of willow, the total offspring included 293 ♀♀, 60 ♂♂. The excess of ♀♀ was due largely to those crosses in which *S. cinerea* participated. A mating of ♀ × ♂ within this species yielded 24 ♀♀, no ♂♂. Offspring of crosses between species other than *S. cinerea*, in general, produced both sexes in approximately equal numbers.

In two of Nilsson's crosses monoecious individuals appeared. *S. viminalis* × *daphnoides* yielded 12 ♀♀, 11 ♂♂, 9 monoecious. The distribution of ♀ and ♂ flowers in the catkins on the monoecious plants was coarsely mosaic; similar plants have been classed by taxonomists as forma *androgyna*. Nilsson considered them sectorial chimeras. He assumed each parent to be heterozygous for a dominant gene tending to dioecism. The offspring homozygous for the recessive gene were monoecious.

The cross (*S. repens* × *viminalis*) × *aurita* yielded 5 ♀♀, 1 ♂, 4 modified ♂♂; in the last-named class (forma *metamorphosans*) some stamens show a variable tendency to develop in a pistil-like fashion, the typically ♂ and the modified flowers being so arranged as to suggest a sectorial chimera. One modified ♂ was monoecious, with ♀ and ♂ flowers so distributed that the plant was considered a periclinal chimera. This monoecious plant mated with an F₁ ♀ produced a progeny of 10 ♀♀, 1 ♂, 7 modified ♂♂ (of which one was monoecious). A recessive gene for modification was assumed, present in the original *aurita* parent and finding expression only in ♂♂. The difference between the *metamorphosans* and the monoecious types appeared not to be genotypic.

Harrison (1924) has noted that *androgyna* forms, like those obtained by Nilsson, are recorded for various *Salix* hybrids. He held them to be genetic ♂♂, but, being heterozygous for sex-tendency genes, and because of their hybridity, their sex-expression is easily modifiable by environmental influences. *Metamorphosans* forms have been reported, according to Harrison, in 6 pure species of *Salix*, and only once (by Nilsson) in a hybrid. Harrison found

metamorphosans plants of certain species to be regularly infested by Eriophyid mites, and concluded that the modified sexual condition here is due to the influence of the parasites.

Cannabis sativa. Contemporaneous with Correns' experiments on *Bryonia* and *Lychnis* were those of Noll (1908), chiefly with hemp. Plants of this species of either sex commonly bear at least occasional flowers of opposite sex as well as hermaphroditic flowers; and the sex-expression is subject to modification by a variety of environmental factors. Previous work (cited by Noll, also by Strasburger, 1900) had shown that the sex ratio among European strains of the species (sufficiently large numbers being counted) is commonly about 100 ♂♂: 115–120 ♀♀. Marked deviations, to be sure, appear in local races.

Among the offspring of individual ♀ plants, abundantly pollinated, Noll found sex ratios ranging from 100:10 to 100:900. From these great differences between the progeny of different mothers, he concluded that the sex of the offspring can not be maternally determined. On the other hand, when the pollen of a single ♂ plant was applied sparsely to the stigmas of various ♀♀, the offspring gave a sex ratio approaching that characteristic of the race. Noll decided that, in relation to sex tendencies, all eggs are alike; ♂ gametes are of two kinds. Thus far he and Correns agreed; but as to the nature of the two kinds of ♂ gametes they differed. From the fact that regeneration from any part of a plant gives rise to plants of the same sex as the parent, Noll concluded that every cell of a ♀ plant, including macrospores and eggs, must carry the ♀ tendency, every cell of a ♂ plant, including microspores and ♂ gametes, must carry the ♂ tendency. The difference between the two types of ♂ gametes, then, is that their ♂ tendencies differ in potency. If a more potent ♂ gamete unites with an egg, the ♂ tendency dominates and the offspring is ♂; if a less potent ♂ gamete functions, the ♀ tendency dominates and the offspring is ♀. Noll's conclusion was supported by Strasburger (1909a, 1910b).

Krüger (1908) found that isolated ♀ hemp plants produced some seeds, which gave rise to ♀ offspring. Finding no flowers on the parent plants, he argued for the occurrence of parthenogenesis.

McPhee (1925) distinguished ♀, ♂ and "intersexual" plants, the latter being chiefly ♀ but bearing some ♂ flowers. Female plants crossed with intersexes, and intersexes selfed, gave all ♀ offspring,

save for 3 ♂♂ in one family which may have been due to stray pollen. Females \times ♂♂ gave approximately equal numbers of ♀ and ♂ offspring. Selfing ♂ intersexes (plants chiefly ♂ but with some ♀ flowers) resulted in a progeny of 3 ♂♂ and one ♀. McPhee concluded, like Correns and Noll, that plants of ♀ type (wholly or chiefly ♀) are homozygous and that male-type plants are heterozygous for sex tendencies. By this time something was known of sex chromosomes in angiosperms, and the question as to whether particular gametes are female- or male-determining had lost much of its significance.

Experiments of Hirata (1927, 1931) with Japanese varieties of hemp gave essentially similar results. He recognized female-type (strictly ♀ and ♀ intersex) and male-type (strictly ♂ and ♂ intersex) plants. Some of his ♂ intersexes bore a few ♀ flowers; others produced, instead, structurally bisexual flowers with abortive pistils. Female-type plants selfed or intercrossed gave female-type progeny; the very rare ♂ offspring may again have been due to stray pollen. Female-type \times male-type gave female- and male-type offspring in about equal numbers. Male intersexes selfed gave a progeny of 6 ♀♀, 2 ♂♂. Some of Hirata's results, particularly an increase in intersexuality from generation to generation when selection was made of individuals showing the higher degrees of this character, suggested that variations in intersexuality observed in different strains have a genetic basis. He conceived that the balance of sex-influencing factors in the X chromosome is in favor of femaleness, in the Y chromosome in favor of maleness. Genetic tendencies toward intersexuality result from variations in potency of the various individual factors.

Hoffmann (1938) has summarized these and other studies of hemp. His own limited experiments suggest that monoecism is recessive to dioecism.

Urtica. Female plants of *U. dioica* bearing occasional ♂ and bisexual flowers, when selfed or intercrossed, were found by Strasburger (1910a) to yield a wholly ♀ progeny.

Negodi's (1929) study of *U. caudata* shows a great variety of forms ranging from monoecious to andro- and gynodioecious. Taking into consideration the proportion of catkins of each sex on individual plants, he finds that in the offspring of a selfed monoecious plant the sex-expression may be stated in a percentage approxi-

mately equal to that of the parent; the offspring of two mated individuals differing in percentage of sex-expression are on the average approximately median in this respect to the parents. Negodi adopts essentially Correns' formula for the transmission of sex tendencies, and assumes for a subdioecious species a varying potency of one or the other sex-tendency factor.

U. cannabina (Negodi, 1931) includes ♀ and monoecious plants. A mating between ♀ and monoecious yielded 19 ♀♀, 17 monoecious. In terms of proportions of ♀ and ♂ catkins, the mother was 100% ♀, the father 85% ♀, 15% ♂. The progeny, taken together, were 94.5% ♀, 5.5% ♂. Negodi thus finds the law based upon his study of *U. caudata* applicable also to a species of somewhat different sex-expression. Of another strain of *U. cannabina*, Negodi (1935a) selected two monoecious plants bearing different proportions of ♂ catkins. Each was selfed as well as mated with purely ♀ plants. The "more male" plant, selfed, gave 58% monoecious offspring (the remainder ♀); crossed with ♀ plants, it gave 12–15% monoecious offspring. The "less male" plant, selfed, gave only 3% monoecious; crossed with ♀♀, 2–7% monoecious offspring. He now assumes for the ♀ parents the formula $MMFF$, for the monoecious parents $MMFF_1$. The latter plants produce gametes MF (determining femaleness) and MF_1 (determining monoecism). The first ("more male") monoecious plant tested produced about 50% of each kind of gamete. The second produced only about 5% of (functional) MF_1 gametes.

Rumex. Raunkiaer (1918) found that in nature as in culture ♀♀ of *R. thyrsiflorus* greatly outnumber ♂♂. He selected two strains, one producing a relatively high, the other a relatively low, proportion of ♀♀. Five ♀ plants of the female-rich strain and 5 of the female-poor strain were divided; one half of each was pollinated from a ♂ of the female-rich, one half from a ♂ of the female-poor strain. The distribution of the sexes among the offspring was found to be determined chiefly, if not entirely, by the mother; the influence of the father in this respect was at most slight.

Certain studies of *R. Acetosa* will be referred to in connection with the discussion of sex chromosomes.

Spinacia oleracea. Nohara (1923) made matings involving two varieties of spinach. In each progeny, ♀♀ and ♂♂ were approximately equal in number. The progeny of a selfed monoecious plant

(Negodi, 1934) consisted of about 90% monoecious, 10% "gynodioecious" plants.

Silene. In *S. Otites*, the sexes are sharply separate, save that a small proportion of the ♂ plants bear bisexual flowers. Such counts as have been made in the wild show ♂♂ in excess of ♀♀. Newton (1931) found in this and related forms also a few apparent ♀♀ which produced ♂ or bisexual flowers. In Correns' (1928) study of *S. Otites*, ♂♂ bearing some bisexual flowers, selfed or intercrossed, produced only ♂ or intersexual ♂ offspring. Females × intersexual ♂♂ yielded ♀♀ and ♂♂, a few of the latter bearing bisexual flowers. In one strain of this species, both petals and stamens were replaced by carpels.

Newton's (1931) and Sansome's (1938) results agree in most respects with Correns'. They used plants discriminated as *S. Otites*, *S. Otites* var. *umbellata*, *S. pseudotites*, and *S. wolgensis*. Matings of ♀♀ × ♂♂ within each of the first three named forms gave about equal numbers of the two sexes. Interspecific or intervarietal crosses, or matings in which one parent was an F_1 hybrid, resulted similarly except that a few individuals classed as ♂♂ appeared. These ♂♂ are considered by Sansome to be modified ("unbalanced") ♀♀. When *S. pseudotites* ♀ was mated with a ♂ of another species, a large preponderance of ♀ offspring appeared—an instance of thelygeny. Between different progenies of ♂♂, either selfed or used as ♀ parents, marked differences appeared (an excess of ♀♀, an excess of ♂♂, or equality of the sexes). Sansome concludes that in *S. Otites* the ♀ is heterozygous (Aa), the ♂ homozygous (AA) for sex-tendency genes or gene complexes. On the other hand, in *S. pseudotites*, as in most dioecious species that have been studied, the ♀ is homozygous (BB), the ♂ heterozygous (Bb). He suggests that the differing conditions in the two species may have developed from a primitive hermaphroditic condition in consequence of different chromosomal changes (such as inversion) combined with gene mutations. The genetic differences between ♂♂ are explained by subsidiary sex factors.

S. Roemerii is further from a strictly dioecious condition than is *S. Otites*. Its plants (Correns, 1928) fall into two classes, ♀ and "± ♂." Those of the latter class bear a variable number of fertile bisexual flowers; in some strains also some ♀ flowers. Varying degrees of divergence from pure maleness in different ± ♂ strains

are in some measure due to genotypic differences, since in general the offspring of a particular $\pm \sigma$ plant resemble the parent in proportion of σ and bisexual flowers.

Female plants pollinated (necessarily) from $\pm \sigma$ plants produce about equal numbers of φ and $\pm \sigma$ offspring. The $\pm \sigma$ plants which produce seeds give rise to progeny also $\pm \sigma$.

Negodi's (1935b) results with *S. Roemerii* are similar. He assigns to $\varphi\varphi$ the formula $MMFF$, to some of the subandroecious ($\pm \sigma$) plants, MMF_1f_1 . The latter, selfed, produce $1 MMF_1F_1 : 2 MMF_1f_1 : 1 MMf_1f_1$; all these offspring are subandroecious but in differing degrees, as shown by their varying proportions of φ and σ or bisexual flowers.

Mercurialis annua. Male plants of this species bear occasional φ flowers; φ plants bear a few σ and, more rarely, bisexual flowers. In addition, plants occur which are classed as monoecious or trimonoecious. Most frequently these latter bear φ flowers at first; later, considerable numbers of σ and bisexual flowers. Less common are plants at first σ , later bearing φ and occasionally bisexual flowers.

Krüger (1908) observed that isolated φ plants, apparently without σ flowers, produced, as in *Cannabis*, some seeds which gave rise to $\varphi\varphi$. He concluded that the results showed the occurrence of parthenogenesis. The rare σ offspring were explained as a result of accidental pollination from an outside source. Later work indicates that Krüger overlooked inconspicuous σ flowers on his φ plants.

Bitter (1909) found that isolated, close-pollinated $\varphi\varphi$ produced offspring chiefly φ (723 $\varphi\varphi$, 21 $\sigma\sigma$). Focke, quoted by Bitter from a personal communication, obtained only φ offspring from isolated $\varphi\varphi$.

In Strasburger's (1909b) work, φ plants pollinated from their own σ flowers or from those of similar plants, gave almost exclusively φ offspring. The two $\sigma\sigma$ obtained were explained by the accidental access of strange pollen. Females $\times \sigma\sigma$ gave a progeny of 40 $\varphi\varphi$, 31 $\sigma\sigma$.

The most extensive study of sex-inheritance in *Mercurialis* is by Yampolsky (1916-1930). In his experiments, $\varphi\varphi$ selfed (pollinated from their own $\sigma\sigma$ flowers) gave all φ offspring. Males selfed gave all σ offspring. Monoecious plants selfed gave all monoecious offspring.

Gillot (1924) obtained from seeds borne by plants "monoecious, predominantly ♂" 17 plants classed as ♂, 4 as ♀, 20 like the parent plants. One form of the species (also distinguished as *M. ambigua*) is constantly more or less intermediate in its sex-expression; the tendency to monoecism (or trimonoecism) is inherited.

Kuhn (1936) whose results have been but briefly reported, agrees with Yampolsky that selfed ♀♀ of *M. annua* give rise to wholly ♀ progeny. Selfed ♂♂, however, contrary to Yampolsky's findings, yield offspring in the proportion of 3 ♂♂ : 1 ♀.

In Negodi's (1937) experiments, "subgynoecious" plants selfed or intercrossed produced 88% ♀♀ (including subgynoecious), 12% ♂♂. Subgynoecious individuals planted with ♂♂ yielded 50% ♀♀, 50% ♂♂.

Sansome (1938) says that the ♀ of *M. annua* is heterogametic. Apparently he is supported in this conclusion by the fact, quoted from Correns, that ♂♂ are slightly more numerous than ♀♀.

Gabe (1939) supplies a possible explanation of the discrepancies in previous work as to the progenies of selfed or inter-crossed ♂♂. He finds two classes of ♂♂, "normal" and "anomalous"; the latter have greenish, non-dehiscent or incompletely dehiscent anthers and apparently much abortive pollen. Matings of ♂ × ♂ gave 179 ♀♀, 296 normal ♂♂, 127 anomalous ♂♂. Selfed ♀♀, as in earlier work, yielded almost exclusively ♀♀ (502 ♀♀, 3 ♂♂). Gabe assumes ♀♀ to possess 2 X chromosomes; normal ♂♂, an X and a Y (not cytologically distinguishable); anomalous ♂♂, 2 Y's. The hypothesis is supported by the facts that ♀♀ × normal ♂♂ yielded 608 ♀♀, 466 ♂♂; ♀♀ × anomalous ♂♂ gave a progeny almost wholly ♂ (13 ♀♀, 659 ♂♂).

Vitis. The extensive literature dealing with sexual conditions in grapes and their behavior in inheritance has been summarized by Negrul (1936), Oberle (1938), and Bethmann (1939). Three types of plants occur: ♀♀ with reflexed stamens producing non-viable or rarely viable pollen (in a very few observed cases, with no trace of anthers); ♂♂ with pistils rudimentary or absent; and, in cultivated races, rare in wild species, functional ♂♀. Other infrequent aberrant types are listed by Negrul.

All wild species of *Vitis* are more or less strictly dioecious. Selection under cultivation has resulted in the establishment of hermaphroditic, and of a smaller number of ♀, varieties; fruiting in the

latter depending necessarily upon the presence in their vicinity of hermaphroditic or ♂ plants. The selected hermaphroditic strains are secondary ♀♀; the genetic behavior of all that have been studied shows them to be mutated ♂♂.

Various intraspecific matings of ♀♀ × ♂♂ (listed by Negrul) have resulted in F_1 progenies consisting of approximately equal numbers of the two sexes.

Hedrick and Anthony (1915) reported that a "hermaphrodite female" mated with a ♂ gave about equal numbers of ♀♀ and ♂♂. Hermaphrodites selfed produced only ♀♀. However, as Valteau (1916) pointed out, Hedrick and Anthony had classed functional ♀♀ as ♀♀. An analysis by Valteau of their matings showed that certain ♀♀ selfed produced only ♀♀; others selfed or intercrossed yielded approximately 3 ♀♀ : 1 ♂. Females selfed or intercrossed, or ♀♀ × ♀♀, produced about equal numbers of ♀♀ and ♀♀. Hermaphrodite × wild ♂ gave ♀♀, ♀♀, and ♂♂. Hermaphrodite × F_1 ♂ gave ♀♀ and ♂♂. Female × F_1 ♂ gave ♀♀, ♀♀, and ♂♂.

Valteau supposes FF to represent the ♀, FM the ♂. But closely linked with F is a suppressed factor for maleness; with M is a suppressed factor for femaleness. If F becomes weakened, or if the linked suppressed factor becomes active, the result is a complex which may be expressed as H . FH then would represent a ♀. Another type of ♀ (HH) may appear (from $FH \times FH$). A second type of ♂ (MH) also is possible.

Müller-Thurgau and Kobel (1924), using cultivated varieties, also found that ♀♀ of certain strains, selfed or intercrossed, produced only ♀♀, whereas those of other strains produced ♀♀ and ♀♀ in approximately a 3 : 1 ratio. Hermaphrodites × ♂♂ produced about equal numbers of ♀♀ and ♂♂. They assumed two pairs of genes regulating respectively the presence or absence of the pistil (K, k) and the functional or non-functional development of stamens (S, s). In wild species the ♀ has the constitution $kkss$, the ♂ $KkSs$. On this basis they held that occasional apparently exceptional cases among offspring are more easily explained.

Kobel (1933) reported similar results in crosses involving European and American cultivated varieties. In addition, he utilized two plants of an intersexual type first described by Stout (1921). These were ♂♂ with some developed and functional pistils. Both intersexes, used as pollen parents, mated with a hermaphroditic

strain, gave a progeny of which about half were ♂♂ and ♀♀, the other half ♂♂ and intersexes. In two other matings unexpected intersexes appeared.

Negrul (1936) assumes that primary and secondary sexual characters are determined by a considerable number of genes borne on a single pair of chromosomes (F and f), hence closely linked in inheritance. The ♀ has two f chromosomes; the ♂, F and f. Small mutations occur frequently in the dominant F chromosome, which affect the development of ovaries and lead to the appearance of intersexes and ultimately of ♂♂. The F chromosome bearing the mutated genes is designated F_n . Hermaphrodites may then be F_nf or F_nF_n . The combination of F_nF may occur in certain matings; this is a ♂ in which hermaphroditism (F_n) is recessive. Negrul recognizes that his hypothesis does not cover all results of inter-specific crosses.

The experiments of Breider and Scheu (1938) showed that among hermaphroditic races of the European *V. vinifera* the same two types of genetic behavior appear that had been described by previous workers. Both sorts of ♂♂ are considered to be secondarily derived from ♂♂ with the allosome formula XY. (X and Y chromosomes have not been recognized cytologically in *Vitis*.) One sort of ♂ retains the XY composition and is hence heterogametic. Such a ♂ selfed or mated with a similar ♂ would be expected to produce 1 XX (♀): 2 XY (♂): 1 YY. The latter (YY) represents the second sort of ♂ which, selfed or intercrossed with a similar ♂, will produce only ♂♂. Females (XX) crossed with XY ♂♂ yield equal numbers of ♀ (XX) and hermaphroditic (XY) offspring. Further evidence that ♂♂ are mutated ♂♂ is found in the occasional appearance of ♂, but not of ♀, flowers on hermaphroditic plants; also in the fact that in certain strains a few ♂♂ occur as a result of selfing YY ♂♂. Some hermaphroditic strains, selfed or crossed with various other ♂♂, give an unexpectedly small proportion (8–14%) of ♀♀. An unknown number of genes on the autosomes (of *V. vinifera*) are assumed, favoring respectively the development of ♂ and of ♀ organs. A sex-tendency gene (α) is borne on the Y chromosome, none on the X chromosome. Hermaphrodites result from the mutation of α to a weaker α' . Plants with two Y chromosomes are (nearly always) ♂♂ because the two α' genes present are not sufficient to swing the

balance toward maleness. The possibility is recognized that ♀♀ , not yet observed, may occur which are modified ♀♀ . Less extensive crosses between *V. vinifera* ♀♀ and *V. riparia* ♂♂ gave results explicable according to these formulae. The results of crossing *V. vinifera* ♀♀ with *V. rupestris* ♂♂ were less consistent, requiring in some instances a polyfactorial interpretation.

Oberle's (1938) matings involved European and American cultivated varieties both hermaphroditic and ♀ , as well as seedlings derived from numerous crosses, some hermaphroditic, some ♀ , and two ♂ . His results, in general harmony with those of earlier workers, lead him to accept the concept of numerous genes affecting sex-expression. Mutations in some of these genes account for unusual forms, including intersexes, which sometimes occur. The basic constitution is thus formulated in terms of closely linked gene-complexes borne on one pair of chromosomes: *Sp* determining, and *sp* inhibiting, normal pollen-development; *so* determining, and *So* inhibiting, normal ovule-development. Females have the constitution $\frac{so\ sp}{so\ sp}$; ♂♂ (ordinarily), $\frac{So\ Sp}{so\ sp}$; ♀♀ , $\frac{so\ Sp}{so\ Sp}$ or $\frac{so\ Sp}{so\ sp}$. The former (homozygous) type of ♀ , selfed, intercrossed, or mated with ♀♀ , yields an entirely hermaphroditic progeny. The second (heterozygous) type of ♀ , selfed or intercrossed, produces 3 ♀♀ :1 ♀ ; mated with ♀♀ , it produces equal numbers of ♀♀ and ♀♀ ; mated with ♂♂ , its progeny is 1 ♀ :1 ♀ :2 ♂♂ . In the latter case, a second type of ♂ may appear with the constitution $\frac{So\ Sp}{so\ Sp}$. Two ♂♂ studied by Oberle seem to have been of this type.

Bethmann (1939), whose experimental results are essentially similar to those of his predecessors, assumes in the primitive hermaphroditic condition two closely linked autosomal genes, *p* and *n*. Independent mutations occurred, of *p* to *P*, which inhibits the development and functioning of male organs, and of *n* to *N*, which similarly affects female organs. The possible combinations on a single chromosome are, then: *pn*, *Pn*, and *pN*. Additional freely assorting autosomal factors, *A* and *B*, strengthen the effect respectively of *P* and *N*.

Carica Papaya. Hofmeyr (1938a, b) finds that matings of $\text{♀} \times \text{♂}$ yield 1 ♀ :1 ♂ ; $\text{♀} \times \text{♀}$, 1 ♀ :1 ♀ ; $\text{♀} \times \text{♂}$, 1 ♀ :1 ♀ :1 ♂ ; ♀ selfed, 2 ♀♀ :1 ♀ ; a ♂ (with occasional bisexual flowers) selfed, 1

♀ : 2 ♂♂. The results are explained by the assumption of an allelic series of 3 genes; ♀♀ have the constitution *mm*; ♂♂, *M₁m*; ♂♂, *M₂m*. The combinations *M₁M₁*, *M₂M₂*, and *M₁M₂* are apparently nonviable. Storey (1938) agrees as to the progeny of ♀ × ♂, of ♀ × ♂, and of a selfed ♂. A selfed ♂ produced 74 ♂♂, 39 ♀♀ (approximately 2 : 1), and 2 ♂♂.

Salvia pratensis. Blaringhem (1932) obtained a marked preponderance of ♀♀ from matings of ♀♀ with an "intermediate."

Valeriana dioica. Male plants with some tendency toward hermaphroditism, close-pollinated, gave (Correns, 1928) wholly ♂ progenies.

Antennaria dioica. In this species, in addition to typical ♀♀ and ♂♂, "aberrant ♀♀" occur which, despite differences in floral structure, are functionally ♀; also, predominantly female ♂♂; and intermediate ♂♂, the structure of whose flowers approaches the ♂ type. To explain the appearance of these forms, Ubisch (1936) assumes for the typical ♀ the formula *FFMM*; for the typical ♂, *FfMM*. He supposes that a mutation has occurred, involving weakening of the ♀ tendency, of the *F* gene to *F'*; and that another mutation, of the *f* gene to *f'*, involved a strengthening of the ♀ tendency. Varying combinations of *F*, *F'*, *f*, and *f'* account for varied sexual conditions. Certain combinations, as *F'fMM*, result in "inframales" which, however, seem to be indistinguishable from typical ♂♂.

Ubisch (1932-1936) finds that matings between certain strains produce a preponderance of ♀♀; other matings result in approximate equality of the sexes; and still others in a preponderance of ♂♂. These results are explained by a fertility gene *S* or *S'*, borne on the same chromosome as the *F*, *F'*, *f*, or *f'* gene. If the fertility gene borne by the pollen tube is similar to both genes present in the style, fertility is highest; if the ♀ (and therefore the stylar tissue) is heterozygotic, fertility is lower; and if both genes in the style are opposite to that of the tube, fertility is still lower, but not necessarily reduced to zero.

Petasites japonicus (Ikeno, 1937) includes plants of two types: one bearing ♀ flowers, the other having flowers structurally bisexual but never producing seeds—hence, functionally ♂. The species is therefore essentially dioecious, although Ikeno treats it as gynodioecious. Seeds borne by ♀ plants (necessarily pollinated from functional ♂♂) gave rise to 21 ♂♂, 14 ♀♀. Among those classed

as ♀, however, some bore occasional heads of ♂ flowers. Ikeno assumes that larger families would show approximate equality of the two sexes.

Cirsium arvense (Correns, 1916, 1928) includes plants purely ♀, others ranging from pure ♂♂ to those with a varying proportion of bisexual as well as ♂ flowers. The pistils of the bisexual flowers, however, are only occasionally functional (with evidence of genetic differences among distinct strains); hence, plants of the second class are in a functional sense chiefly or entirely ♂. A progeny derived by Correns from fruits of these ♂♂ consisted of 8 ♀♀, 9 ♂♂. In later similar experiments with other ♂ plants, the offspring were all ♂♂. Female plants of *C. arvense* pollinated from the gynodioecious *C. oleraceum* and *C. palustre* produced only ♀ offspring.

The results cited in preceding pages indicate that in many "subdioecious" species ♀ plants which, possessing occasional ♂ flowers, can be selfed or intercrossed produce exclusively or almost exclusively ♀ offspring. An apparent exception is seen in Hedrick and Anthony's work with *Vitis*. The rare ♂♂ sometimes appearing in other cases may have been due to extraneous pollination. Male and chiefly ♂ plants, when they can be close-pollinated, yield both ♂ and ♀ offspring. Exceptions to this rule appear to be *Silene Otites*, *S. Roemerii*, and *Valeriana dioica*. It appears that in general, as in *Bryonia* and *Lychnis*, ♀♀ are homozygous, ♂♂ heterozygous for one pair of genes (corresponding to Correns' realizators) concerned in sex-expression. It does not follow, however, that this allelic pair must always be the same. Indeed, since dioecism has obviously arisen independently in various lines, the contrary is more probable. Hermaphroditic plants in these species, when selfed or mated with ♀♀, in general prove to be, like ♂♂, heterozygous.

If ♂♂ are ordinarily heterozygous for one pair of genes (designated, e.g., as *M*, *m*), the selfing or intercrossing of ♂♂ (*Mm* × *Mm*) when this is possible would be expected to yield three classes of offspring: *Mm* (♂), *mm* (♀), and *MM*. The third class seems to be represented in certain cases, as in that of Gabe's anomalous *Mercurialis* ♂♂. In general, as in *Carica*, this class is probably non-viable. A similar expectation as to secondary ♂♂ which, as in most cases studied, are mutated ♂♂, is justified by the occurrence in *Vitis* of two types of ♂♂.

But the occurrence of so many modifications of strict dioecism, leading to the extremes of hermaphroditism (*Vitis*) and monoecism (*Mercurialis*), indicate that, in addition to the one determinative pair of genes clearly suggested, many others are operative in sex-determination. Although numerous suggestions as to their nature have been advanced, in no dioecious species has genetic analysis proceeded far enough to furnish an adequate conception of the number of genes concerned.

While in many dioecious angiosperms the ♂ is shown to be heterozygous in terms of one determinative allelic pair, it remains possible that in some species the heterozygous sex is the ♀. This, it has been seen, is suggested by Sansome for *Silene Otites* and *Mercurialis annua*. In the genus next to be mentioned, heterozygosis in the ♀ is clearly established.

Fragaria. Although the sexual condition varies greatly among strawberries, a number of wild species have been shown to be dioecious (Valleau, 1918), as are some of the cultivated varieties (known as *F. grandiflora*) which seem to derive from hybrids between *F. Chiloensis* and *F. virginiana*. There are hermaphroditic wild species and hermaphroditic cultivated varieties (with a tendency to trimonoecism) as well as hermaphroditic individuals among dioecious species and varieties. The hermaphroditic cultivated forms, Valleau (1918) and Kuhn (1930a) agree, are mutated ♂♂. In individuals of either sex in a dioecious race, the organs of the opposite sex are represented by structures ranging from mere rudiments to nearly complete development. As a rule, some of the pistils borne by ♂ plants are functional; hence there is no sharp distinction between ♂♂ and ♀♀.

The various species are respectively diploid ($n=7$), hexaploid ($n=21$), and octoploid ($n=28$). Some of the crosses referred to below were between species of different chromosome numbers. Such differences inhibit the success of many crosses; in others, they cause the partial or complete sterility of the hybrids. Thus far, however, save in certain of Lilienfeld's experiments, it does not appear that the morphological sex-expression of hybrids is influenced by these differences, so far as the hybrids are able to develop to the stage of flower-production.

Richardson's (1914-1923) experiments involved what were identified as 8 species. Matings of ♀♀ × ♂♂ gave progenies about

half ♀♀, half ♂♂ and ♂♂ (in most cases the latter two classes were grouped together as "♂ or ♀"). A ♀ mated with a ♂ produced 20 ♀♀, 14 ♂♂. A ♂ selfed produced 29 ♂♂, 7 classed as ♀♀; but some of the latter in the second year proved more or less hermaphroditic. A nearly pure ♂ used as pistil parent, pollinated from a ♀, gave a majority of ♂♂, a minority of ♂♂.

The early work of Anthony (1917) is difficult of interpretation because he distinguished only between "perfect" (hermaphroditic) and "imperfect" (♀ and ♂) plants. Apparently his results resembled those of later writers.

Valleau (1923) worked with cultivated varieties. Hermaphrodites selfed produced only (structural) ♂♂ which varied greatly in fertility. A few offspring ("somatic" ♂♂) in 3 families were completely female-sterile, hence functionally ♂. F_1 hybrids were used as parents in some of these matings, without modification of the general nature of the results. Hermaphrodites intercrossed likewise produced only ♂♂, save that in two families a few apparent ♀♀ (probably really ♂♂) appeared. A ♂ plant whose single ♀ flower was pollinated from a ♂ plant gave a progeny of somatic ♂♂. Females × ♂♂ produced nearly equal numbers of ♀♀ and ♂♂.

In these results with *Fragaria* it appears that, differently from what has been seen in many other dioecious species, ♂♂ and ♂♂, selfed or intercrossed, produce essentially one class of offspring. Valleau was the first to conclude that the ♀ (*FM*) is heterozygous, the ♂ (*MM*) homozygous. He assumed that closely linked with the *F* gene is a suppressed factor for maleness; closely linked with the *M* gene, a suppressed factor for femaleness. In hermaphroditic races (derived from ♂♂) the ♀ factor is not suppressed, and the linked factors are represented by *H*, recessive to *F*, dominant to *M*. A ♂ then might be genetically *HH*. If such a ♂ is mated with a ♀, the offspring are half *FH* (♀♀) and half *HM* (♂♂). A ♂ of the latter type selfed would give 1 *HH*:2 *HM*:1 *MM*. The *HH* and the *HM* ♂♂ would differ in fertility. The *MM* class, Valleau thought, was represented in his experiments by the somatic ♂♂ which were functionally ♂.

Kuhn (1930a) modifies Valleau's formula by adopting Correns' conception of *A* and *G* genes (for ♂ and ♀ potencies respectively), replacing Valleau's "suppressed factors." The ♀ has the genetic formula *AAGGZZαγ*; the male, *AAGGZZαα*. A weakening of

the α gene leaves the ♀ (with the dominant γ gene) unchanged, but allows the G genes in the ♂ to come to expression; some structurally bisexual flowers appear on the ♂ plant. If the potency of the α gene is reduced to zero, the plant (with the $AAGG$ complex) is wholly hermaphroditic.

In Correns' (1928) cross of a cultivated hermaphroditic race with ♂ *F. elatior*, the progeny were all ♂. *F. elatior* ♀ × *elatior* ♂ produced about equal numbers of ♀♀ and ♂♂.

A cross made by Mangelsdorf and East (1927) of *F. virginiana* ♀ × *F. elatior* ♂ yielded a progeny of ♀♀ and ♂♂.

The results of Schiemann (1930, 1931) agree substantially with those of Richardson and Valleau. Some of her experiments dealt with F_2 and F_3 generations. Much attention was paid to the differing degrees of fertility as manifested respectively by ♀♀, ♂♂, and ♂♀. She also ascribed the difference between structural ♂♂ and structural ♂♀ to differences in the valency of a sex-tendency factor. Separate sterility factors determine the various grades that intervene between the condition of complete structural and physiological maleness and that of perfect hermaphroditism. Factors of another set influence the degree of sterility or fertility of offspring. Sex-expression of ♀♀, while modifiable by environmental conditions, is more stable than that of ♂♀. Apart from environmental effects, mutations occur which affect sex-expression in either sex. Full fertility is dominant; hence, a fully fertile ♀ may be either homozygous or heterozygous for a pair of fertility-sterility factors.

Chodat (1930, 1933) found also that a mating of ♀ × ♂ produced nearly equal numbers of ♀♀ and ♂♀. F_1 ♂♀ selfed produced only ♂♀. Other F_1 ♂♀ × a *virginiana* ♂ produced a progeny all hermaphroditic but with carpels, while still functional, reduced in number and size.

Lilienfeld (1933–1936b), like Schiemann, has carried *Fragaria* crosses to the F_2 and F_3 generations. He began by mating a ♀ of *F. elatior* (hexaploid) with *F. nipponica* (diploid, hermaphroditic). The offspring were ♀ and ♂ in nearly equal numbers, as were those from a mating between *F. elatior* ♀ and *elatior* ♂. However, when the F_1 ♀♀ and ♂♂ from the interspecific cross were mated, a portion of the progeny were ♂♀ (in addition to ♀♀ and ♂♂). F_2 ♀♀ × F_2 ♂♂ gave similar results; here the ♂♀ approached 25% of the progeny. If F_2 ♀♀ or *elatior* ♀♀ were mated with F_2 ♂♀, the progeny

likewise included ♀♀, ♂♂, and ♀♂. F_2 ♀♀ \times F_3 ♂♂, or F_2 ♀♀ selfed, produced ♂♂ and ♀♀. Matings between F_1 ♀♀ or ♂♂ and *F. elatior*, and between F_1 ♀♀ or ♂♂ and *F. nipponica* gave ♀♀, ♂♂, and some ♀♂.

Lilienfeld distinguishes different grades of maleness (based on the degree of development of pistils) and different grades of hermaphroditism (according to proportions of fruits set). He considers that *F. nipponica* is a primary ♀, possessing a pair of genes for the hermaphroditic tendency. *F. elatior*, with 6 sets (3 pairs) of homologous chromosomes, bears 6 genes allelic with the hermaphrodite genes of *F. nipponica*; 4 of these represent the ancestral hermaphroditic tendency; but 2 (on one pair of chromosomes) in the ♀ have mutated respectively to the conditions α (for the ♂ tendency) and γ (for the ♀ tendency); in the ♂, both are α . In the interspecific cross, the F_1 offspring have either γ (♀) or α (♂), paired in either sex with a hermaphrodite gene. Among the offspring of these mated F_1 ♀♀ and ♂♂ about 25% of ♀♀ are expected. Actually in the F_2 a smaller proportion appeared; the explanation offered is that some of the excess ♂♂ were genetic ♀♀. In the F_3 , when selected F_2 ♂♂ were used as parents, the proportion was nearer that expected. Some tendency appeared for ♂♂ to revert toward hermaphroditism.

The variable number of α genes possible in the recombinations made is taken to account for the various degrees of "maleness" observed.

It is suggested that certain genes affect the capacity for seed- and fruit-development in ♀♀, and produce still more marked effects in ♀♂.

The self-fertility of *elatior* plants, contrasted with the self-sterility of those of *F. nipponica*, is ascribed to a dominant self-sterility (self-incompatibility) gene, possibly one of an allelic series, in the former species. It should be noted, however, that genes in this category in better-known cases (see the discussion by Stout, 1938), affect the relations between stigma and pollen-tube growth, and so are outside the limits of the present discussion.

Schiemann's (1937) cross of *F. elatior* (hexaploid) ♀ \times *collina* (diploid) ♀ resulted similarly to those of Lilienthal with the cross *elatior* \times *nipponica*.

Thalictrum. A species whose genetic behavior with respect to

sex has been thought to resemble that of the strawberry is *T. Fendleri*. It includes ♀ and ♂ plants, both bearing rare flowers with a suggestion of hermaphroditism, and others which are subandroecious (\pm ♂) to hermaphroditic. In Kuhn's (1936) experiments, most subandroecious and hermaphroditic plants, selfed, produced progenies consisting of ♀♀ and ♂♂ in a ratio of 1:3. Fifty of the F_1 ♂♂ were mated with ♀♀. Of the resulting progenies, 35 included ♀♀ and ♂♂; 15 were exclusively ♂♂. Kuhn concluded that the F_1 ♂♂ are of two classes; two thirds are heterogametic for sex-tendency factors, one third homogametic for a male-tendency factor. The 15 ♂♂ classed as homogametic (whose offspring were all ♂♂) evidently furnish another case of arrhenogeny capable of explanation by Correns' assumption of a lethal factor that prevents the appearance of female offspring. In Kuhn's (1930b) earlier work, crosses were made between females of *T. Fendleri* and the hermaphroditic species *T. foetidum*, *Delavayi*, and *aquilegifolium*. All offspring were ♀♀, but with a marked tendency toward maleness as shown by a reduction in number of carpels. In the cross with *T. aquilegifolium*, carpel-reduction went so far as to result in almost purely ♂ plants. Kuhn's provisional explanation assumed homogamety in the ♀ and a "male-determined" cytoplasmic influence. In another report (1931) he noted that "further investigations make it highly probable that in *Thalictrum Fendleri* heterogamety of the ♀ occurs." Apparently this opinion was later modified.

Morus alba. In the mulberry Schaffner (1925, 1929) found that a few branches of ♂ trees bearing ♀ flowers produced seeds. Of the 16 plants derived from these seeds which grew to the flowering stage, 7 were ♀♀, 4 ♀♀ with a few ♂ flowers, 5 nearly or quite pure ♂♂. Later (Schaffner, 1936), seeds produced by a selfed ♀ plant with a few ♂ flowers gave rise to a similar progeny; 5 pure ♂♂, 4 nearly pure ♂♂, 8 pure ♀♀, one nearly pure ♀, and 2 of mixed sexual character. The conditions seem to differ from those characterizing any of the dioecious species previously mentioned.

SEX CHROMOSOMES IN DIOECIOUS SPECIES

✓ The first definite recognition of sex chromosomes (allosomes) in an angiosperm was by Santos (1923), who described an unequal (XY) pair of chromosomes in ♂ plants of *Elodea gigantea*. However, in the same month as Santos' paper appeared one by Kihara

and Ono (1923a) announcing the presence of a tripartite chromosome complex in pollen mother cells of *Rumex Acetosa*; and at the meeting of the British Association for the Advancement of Science in the previous year Blackburn and Harrison (1923) had reported that "some evidence exists of the presence of an unequal chromosome pair in the ♂ plants" of species of *Salix* and *Populus*. Shortly after, Blackburn (1923) announced the occurrence of an XY pair in *Lychnis alba*; Kihara and Ono (1923b) gave further and conclusive details regarding the sex chromosomes of *Rumex*; and Winge (1923) described XY pairs in *Lychnis alba*, *Humulus japonicus*, and *H. Lupulus*, and an unpaired X in *Vallisneria spiralis*. Winge later changed his views regarding *Vallisneria* and *Humulus japonicus*; and with regard to *H. Lupulus* there is still disagreement. Winge (1932) considers that the X chromosome in *Lychnis* is the equivalent of two Y's. This is on the assumption, supported by others, that the X is the larger; Blackburn (1924) and Warmke and Blakeslee (1939), however, have held that the Y is larger than the X.

These discoveries furnished a corroboration of the conclusion, based on genetic evidence, that one sex (usually the ♀) is homozygous, the other heterozygous, for at least one pair of genes determinative for sex-tendencies—perhaps, as suggested by Winge (1932), because these are epistatic to other genes affecting sex-expression. That homo- and heterozygosis in terms of the postulated genes are related to the visible homo- and heterozygosis in terms of chromosomes is beyond question; although the precise nature of the relation is still unsettled.

A visible difference between the chromosomes of a pair (or within a group of 3 or 4) does not necessarily accompany dioecism. For example, whereas the allosomes of *Lychnis alba* and *L. dioica*, despite the earlier negative results of Strasburger and Sykes, have proved to be among the most conspicuous and easily demonstrable of such structures, in *Bryonia dioica*, whose genetic behavior with respect to sex parallels that of *Lychnis*, careful search has failed to demonstrate an unequal pair. Such failure, of course, does not negative the conclusion from genetic evidence that a pair of determinative genes are present, and that there is therefore a pair of functional sex chromosomes. In this connection it may be added that further study may in some instances disclose small chromosomal differences thus far overlooked.

Tables 1 and 2 summarize the reports to date as to the presence or absence of sex chromosomes in dioecious species and varieties. In their preparation the earlier lists of Kihara (1929*b*), Sinotô (1929*b*), and Lindsay (1930) have been freely used; the literature of the past 10 years has of course provided material additions. In the majority of cases, for obvious reasons, the search for allosomes has been limited to the meiotic divisions in microspore mother cells. When results have been negative, the possibility remains that, as seems to be the case in *Fragaria*, it is the ♀ that is heterogametic. Because of the comparative difficulty in finding the meiotic figures in megaspore mother cells, it must require many years to explore all such possibilities. Incidentally, Meurman's conclusion as to the absence of sex chromosomes in *Ribes orientale* (table 2) is based upon a study of the ♀ plant only, in connection, however, with his failure to find such bodies in the ♂♂ of other dioecious members of the genus.

Certain discrepancies between the present and one or other of the previous lists may be noted.

Rumex Acetosella, for reasons cited below, is included among the species without recognizable sex chromosomes. *Fragaria elatior*, likewise discussed on a later page, is included among those having such structures. Reference is omitted to Meurman's examination of *Tamus communis*. In a preliminary note (1925*a*), he seemed to imply that this species has no unequal chromosome pair, but in his fuller account (1925*b*) he expressed himself as unable to decide upon the question.

In cases of difference of opinion as to the presence of sex chromosomes in a particular species, positive results are here provisionally accepted and the species is listed in table 1, references being given to statements on both sides. Often, though not invariably, it was earlier workers who failed to recognize the allosomes, later students who found them. Instances are the negative reports of Meurman and Håkansson on *Salix Caprea*; of Strasburger and McPhee on *Cannabis sativa*; of Strasburger and Sykes on *Lychnis dioica*; of Newton on *Silene Otites*; of Darling on *Acer Negundo* (regarding which species, however, Sinotô is not entirely certain); and of Lorz on *Spinacia tetrandra*.

A few papers previously cited as showing the absence of allosomes in particular species are not here included. These are reports

which fail to indicate that the problem was under consideration; most of them appeared before sex chromosomes had been recognized in angiosperms.

Citation is omitted also of *Polemonium coeruleum* and *Cucurbita Pepo*, listed by Sinotô, the former after Winge (1923), the latter after Sykes (1909). Neither plant is dioecious, and there is no present reason to expect the occurrence of anything of the nature of sex chromosomes in non-dioecious species. It is true that Pastrana (1932) has reported the presence of an allosome in the monoecious *Begonia Schmidtiana*. According to her description, whereas all other parts of the sporophyte possess 13 chromosomes, the ♂ flower has but 12, apparently in consequence of an unobserved differential mitosis. In meiosis in the macrospore mother cell an unpaired chromosome passes to the micropylar pole; ultimately, then, the 2 micropylar macrospores receive 7 chromosomes each, the chalazal macrospores 6 each. The embryo sac develops from a spore with 7 chromosomes. Her figure 65, however, seems to indicate that the functional macrospore lies at the chalazal end of the row of 4, and should, therefore, have but *six* chromosomes. In view of this and other discrepancies, Pastrana's report, as Mermiński (1936) has pointed out, can not be accepted without confirmation.

As to species nomenclature, Gray's "Manual" has been followed so far as possible; for species not therein included, the usage of the Index Kewensis, again so far as possible, is accepted. When an author has used a different specific name, his synonym is given in parentheses.

Certain cases should be specially mentioned in which uncertainty or difference of opinion exists.

Zanthoxylum piperitum. In pollen mother cells Sinotô (1929b) described a large univalent chromosome which he interpreted tentatively as a lone X; in which case the allosome formula would be: ♀, 2 X; ♂, X. He often saw also, however, an apparently unequal pair. Nakajima (1937) observes an unequal pair and a *small* univalent. The species is included in table 1 among those showing the XX-XY condition; further study is needed.

Dioscorea. As tables 1 and 2 indicate, the statements as to members of this genus are confusing. Strasburger (1909a) found no sex chromosomes in an undetermined species. In *D. sinuata*, Meur-

man (1925a, b) concluded tentatively that the ♂ has 35 chromosomes; in the heterotypic division 18 go to one pole, 17 to the other. In pollen mother cells of *D. caucasica*, however, he found 10 equal chromosome pairs. Smith (1937) counted 81 chromosomes in root tips of *Dioscorea alata*, and 61 in those of *D. reticulata*. He notes that the odd numbers may indicate for these species also the presence of an unpaired X chromosome. Nakajima (1937) reports the presence of an XY pair in pollen mother cells of *D. gracillima* and of *D. Tokoro*.

If Meurman's conclusion regarding *D. sinuata* and Smith's suggestions as to certain other species prove correct, these members of the genus resemble certain insects in having the "XX-XO" type of allosome complement. This condition is at most rare in angiosperms; Sinotô's view as to its occurrence in *Zanthoxylum* was noted above. Winge's (1923) report of the same condition in *Valisneria spiralis* was later found by him (1927a) and by Jørgensen (1927) to be erroneous.

Humulus. Winge (1923) at first described ♂ plants of both *H. japonicus* and *H. lupulus* as showing an unequal chromosome pair. Later it was agreed by Kihara (1928, 1929a, b), Winge (1929a, 1932), Sinotô (1929b), Tuschnjakowa (1929, 1930), and Kihara and Hirayoshi (1932) that the ♀ of *H. japonicus* has 16, the ♂ 17 chromosomes; that in the heterotypic division in pollen mother cells a tripartite chromosome complex appears; and that this complex divides so that either the median chromosome passes to one pole, the two terminal ones to the opposite pole, or one terminal and the median chromosome pass to one pole, the other terminal chromosome to the opposite pole. Winge holds that the three chromosomes of the complex are equivalent (X's); that sometimes two of them are so closely associated as to seem a single large chromosome, and that the ♀ has 2 X, the ♂ 3 X. The Japanese authors maintain that the typical method of division of the tripartite complex is such that the median chromosome (X) passes to one pole, the two terminal chromosomes (Y_1 and Y_2) to the opposite pole; other methods of separation are exceptional and probably abnormal. Kihara finds that the X has two equal arms, each of the Y's one long and one short arm. Kihara and Hirayoshi have observed that in the heterotypic prophase a triradial conjugation occurs, indicating that each arm of the X is at least partially homologous with the longer arm

of one Y. Their formulae would be: ♀, $2X$; ♂, $X + Y_1 + Y_2$. Tuschnjakowa likewise finds the X to be symmetrical, the two Y's asymmetrical. But she holds that the chain-arrangement of X, Y_1 , and Y_2 is variable, and that the separation of the chromosomes is haphazard. In consequence, varying combinations of X's and Y's appear in the sporophytes, although apparently the ♀ always has 2 of these allosomes, the ♂ always 3. In principle, her conclusion as to sex-determination is similar to that of Winge.

As to *H. lupulus*, Winge (1929a, b, 1932) has adhered to his conception of an unequal pair in the ♂; although he considers the larger of the pair (Y), which is constricted in the middle, as equivalent to two X's, and hence to two members of the tripartite complex in *H. japonicus*. Sinotô (1929a, b) finds a tetrapartite complex ($X_1 Y_1 X_2 Y_2$) in *H. lupulus*, the allosome formula being: ♀, $2X_1 + 2X_2$; ♂, $X_1 + X_2 + Y_1 + Y_2$. His conclusion is corroborated (for var. *cordifolius*) by Ono (1937) and Nakajima (1937). Winge holds that Sinotô's tetrapartite complex is a chain of 4 autosomes and that he overlooked the X-Y pair. Sharp (1934) suggests that Sinotô's chain of 4 may be comparable with the chain of 5 chromosomes seen by Kihara (1929c) in one ♂ plant of *H. japonicus*; in the latter case one pair of small autosomes is attached to the tripartite sex-chromosome complex. In table 1, the formulae of the Japanese investigators for both species have been included, although hesitantly, because the present weight of evidence seems on the whole to favor their interpretation.

Phoradendron. Billings (1932, 1933) reports for two species of *Phoradendron* a condition unique, so far as now known, among both plants and animals. He describes an unpaired chromosome (best interpreted probably as a Y) in the ♂, which has in all 21 chromosomes. The odd chromosome passes to one pole in the heterotypic division; microspores then receive 10 and 11 chromosomes respectively. The ♀ has 20 chromosomes.

Rumex. The tripartite chromosome complex occurring in pollen mother cells of various dioecious species of this genus was first described (Kihara and Ono, 1923a, b) for *R. Acetosa*. This complex consists of an X chromosome to either end of which is attached a Y (Y_1, Y_2). In the heterotypic division, X goes to one pole, Y_1 and Y_2 pass to the other pole. Y_1 is somewhat larger than Y_2 ; the X is larger than either Y. The ♀ has two X's. The allosome for-

mula for the species is, therefore: ♀, $2X$; ♂, $X + Y_1 + Y_2$. In the years since 1923 the findings of Kihara and Ono have been abundantly confirmed (table 1). Exceptionally (Sinotô, 1924), the tripartite complex lies in such a position in the heterotypic equatorial plate that its division may occur in other than the usual manner. Such exceptional segregations perhaps explain some of the unusual chromosomal conditions that, as will appear, have been much studied in this species.

The case of *R. Acetosella* remains obscure. Meurman (1925a, b) found in the ♂ 41 chromosomes, 3 of which form in the heterotypic equatorial plate a tripartite chain similar to that in *R. Acetosa*, and were therefore identified as sex chromosomes. However, according to his description, differently from the conditions in *R. Acetosa*, the chain divides in such a way that one terminal chromosome passes to one pole, the median and second terminal chromosomes pass to the opposite pole. Kihara (1925, 1927) found the chromosome number to vary in ♂ plants from 41 to 43, 42 being commonest. He found 42 chromosomes in one ♀ plant, Ono (1930a) 41 in an intersexual plant. Kihara observed that certain chromosome pairs frequently conjugate in a triradial or tetradial manner, the result being the formation of rings of 4 or 6 chromosomes which separate equally in the heterotypic metaphases. In one plant with 41 chromosomes, the odd element appeared as a univalent on the heterotypic equatorial plate and passed undivided to one pole; the daughter groups therefore received respectively 20 and 21 chromosomes. He concluded that Meurman's tripartite chain corresponds to a ring of 4 with a chromosome of one pair lacking; and that there is therefore no evidence as to which chromosomes carry the sex-differentiating factors. In view of Kihara's findings, *R. Acetosella* is included (table 2) in the list of dioecious species without recognizable sex chromosomes.

Atriplex hymenelytra. Billings (1934) describes in pollen mother cells a tripartite complex of which two adjoining elements pass to one pole, the third moving to the opposite pole. He considers the two adherent chromosomes as X's, the third as a Y. The chromosomal constitution of the ♀ plant is unknown. Some of Billings' figures may be taken to represent a single deeply constricted X chromosome rather than two distinct bodies. On this view, *Atriplex* would present the more common XX-XY condition.

Fragaria. After some years of uncertainty (1926, 1929b) due to the difficulty of the material, Kihara finally (1930) concluded that in *F. elatior* there is an unequal chromosome pair in the ♀, no such pair in the ♂. His conclusion is in harmony with the experimental findings already cited which indicate that in this and in other strawberries the ♀ is heterozygous for a pair of sex-tendency genes.

TABLE 1
ANGIOSPERMS: SEX CHROMOSOMES REPORTED PRESENT

Species	References
Female, 2A+2X; Male, 2A+X+Y	
SALICACEAE	
¹ <i>Populus balsamifera</i> L.	Meurman, 1925b
<i>P. Eugeni</i> Dode	Blackburn, 1929
<i>P. generosa</i>	Blackburn, 1929
<i>P. serotina</i> Hort.	Blackburn, 1929; Nakajima, 1937
<i>P. Simonii</i> Carr.	Meurman, 1925b
<i>P. tremula</i> L.	Blackburn and Harrison, 1924
<i>P. tremuloides</i> Michx.	Erlanson and Hermann, 1927
<i>P. trichocarpa</i> Torr. & Gray	Meurman, 1925b
² <i>Salix Andersoniana</i> Sm.	Harrison, 1926
<i>S. aurita</i> L.	Harrison, 1926
<i>S. babylonica</i> L.	Nakajima, 1937
<i>S. bakko</i> Kimura	Nakajima, 1937
<i>S. Caprea</i> L.	Meurman, 1925a; Håkansson, 1929; Nakajima, 1937
<i>S. cinerea</i> L.	Harrison, 1926
<i>S. gracilistyla</i> Miq.	Sinotô, 1929b
<i>S. glandulosa</i> Seemen	Nakajima, 1937
<i>S. japonica</i> Thunb.	Sinotô, 1925, 1928, 1929b
<i>S. leucopithecia</i> Kimura	Sinotô, 1928, 1929b
<i>S. lucida</i> Muhl.	Harrison, 1926
<i>S. melanostachys</i> Makino	Sinotô, 1928, 1929b
<i>S. purpurea</i> L.	Harrison, 1924
<i>S. repens</i> L. (<i>S. integra</i> Thunb.)	Nakajima, 1937
<i>S. sachalinensis</i> F. Schmidt	Sinotô, 1928, 1929b; Nakajima, 1937
<i>S. viminalis</i> L.	Blackburn and Harrison, 1924; Harrison, 1924
var. <i>yezoensis</i> Schn.	Sinotô, 1925, 1928, 1929b

¹ According to Index Kewensis, *P. serotina* Hort. = *P. angulata* Ait. = *P. monilifera* Ait. Bailey (1924) makes *P. monilifera* Ait. = *P. balsamifera* L. (the cottonwood); other authors, including Gray, apply the name *P. balsamifera* L. to the balsam poplar (*P. Tacamahacca* Mill.). *P. Simonii* Carr. is referred by Index Kewensis to *P. balsamifera* L. However, Meurman found marked differences between the two forms thus named as to chromosome behavior during meiosis. He concluded that his *P. Simonii* is a hybrid.

² Index Kewensis makes *S. Andersoniana* Sm. a synonym of *S. nigricans* Sm. Linton (1913) treats the latter name as *nomen confusum* and accepts *Andersoniana*. According to Index Kewensis, *S. japonica* Thunb. = *S. babylonica* L. The two are treated as distinct by Nakajima.

MORACEAE

✓ *Cannabis sativa* L.

Strasburger, 1909a, 1910b; McPhee, 1924; Hirata, 1924, 1929; Sinotô, 1928, 1929b; Breslawetz, 1932; Driga (cited by Hoffman, 1838); Mackay, 1939

Cudrania triloba Hance

Sinotô, 1928, 1929b

Morus bombycis Koidz.

Sinotô, 1925, 1928, 1929b

URTICACEAE

✓ *Urtica dioica* L.

Meurman, 1925a, b

SANTALACEAE

Buckleya Joan Makino

Nakajima, 1937

CHENOPODIACEAE

✓ *Spinacia tetrandra* Stev.

Lorz, 1937; Araratjan, 1939

CARYOPHYLLACEAE

✓ *Lychnis alba* Mill. (*Melandrium album* (Mill.) Garcke)

Blackburn, 1923, 1924, 1928, 1929; Winge, 1923; Heitz, 1925a, b, 1926; Meurman, 1925b; Schürhoff, 1925; Lindsay, 1929, 1930; Breslawetz, 1929

var. *glabrum*
✓ *L. dioica* L. (*M. rubrum* Garcke)

Blackburn, 1929
Strasburger, 1909a, 1910b; Sykes, 1909; Blackburn, 1924, 1928, 1929; Heitz, 1925a, b; Meurman, 1925b; Schürhoff, 1925

L. divaricata Reichb. (*M. divaricatum* Fenzl)

Blackburn, 1928, 1929

L. glutinosa (Rouy) (*M. glutinosum* Rouy)

Blackburn, 1928, 1929

✓ *Silene Otites* Sm.

Blackburn, 1928, 1929; Newton, 1931

MENISPERMACEAE

Cocculus trilobus DC.

Nakajima, 1937

CRASSULACEAE

Sedum roseum (L.) Scop. (*S. Rhodiola* DC.)

Levan, 1933

RUTACEAE

Zanthoxylum piperitum DC.

Sinotô, 1929b; Nakajima, 1937

DAPHNIPHYLLACEAE

Daphniphyllum macropodum Miq.

Sinotô, 1928, 1929b

EMPETRACEAE

Empetrum nigrum L.

Hagerup, 1927

ACERACEAE

Acer Negundo L.

Darling, 1909; Sinotô, 1929b

DATISCEAE

Datisca cannabina L.

Sinotô, 1928, 1929b

VALERIANACEAE

Valeriana dioica L.

Meurman, 1925a, b

CUCURBITACEAE

Trichosanthes cucumeroides

Maxim.

Nakajima, 1937

T. japonica Regel

Sinotô, 1925, 1928, 1929b; Sugimoto, 1928; Nakajima, 1937

HYDROCHARITACEAE

- ³*Elodea canadensis* Michx. Santos, 1924
E. densa (Planch.) Casp. (*E. canadensis* var. *gigantea*; *E. gigantea*) Santos, 1923
Hydrilla verticillata Presl Sinotô and Kiyohara, 1928; Sinotô, 1929b

PALMAE

- Trachycarpus excelsus* Wendl. Sinotô, 1929b
T. Fortunei (Mak.) (*T. excelsus* var. *Fortunei*) Sinotô, 1928, 1929b

LILIACEAE

- Smilax China* L. Nakajima, 1937
S. hederacea L. var. *Nipponica* Nakajima, 1937
 Maxim
S. Oldhami Miq. Nakajima, 1937

DIOSCOREACEAE

- Dioscorea gracillima* Miq. Nakajima, 1937
D. Tokoro Makino Nakajima, 1937

Combinations other than $2A + 2X$, $2A + X + Y$

MORACEAE

- Humulus japonicus* Sieb. & Zucc.
 (♀, $2A + 2X$; ♂, $2A + X + Y_1 + Y_2$) Winge, 1923, 1929a, 1932; Kihara, 1928, 1929a, b; Sinotô, 1929b; Tuschnjakowa, 1929, 1930; Kihara and Hirayoshi, 1932
H. Lupulus L.
 (♀, $2A + 2X_1 + 2X_2$; ♂, $2A + X_1 + X_2 + Y_1 + Y_2$) Winge, 1923, 1929a, b, 1932; Sinotô, 1929a, b
 var. *cordifolius* Maxim. (same chromosome complement) Ono, 1937; Nakajima, 1937

LORANTHACEAE

- (♀, $2A$; ♂, $2A + Y$)
Phoradendron flavescens Nutt. Billings, 1932, 1933
 var. *macrophyllum* Engelm. Billings, 1932, 1933
P. villosum Nutt.

POLYGONACEAE

- (♀, $2A + 2X$; ♂, $2A + X + Y_1 + Y_2$)
⁴*Rumex Acetosa* L. Kihara and Ono, 1923a, b, 1925; Sinotô, 1924; Ono, 1926, 1928; Kihara and Yamamoto, 1931
 var. *haematinus* Kihlman Jaretsky, 1928
 var. *pratensis* Wallr. Jaretsky, 1928
R. arifolius All. Kihara and Ono, 1926; Jaretsky, 1927, 1928

³ *Elodea* is now designated (at least by American taxonomists) as *Anacharis*.

⁴ Index Kewensis makes *R. arifolius* All. (so cited by Kihara and Ono and by Jaretsky) = *R. montanus* Desf. *R. arifolius* L., however, is a distinct species. Index Kewensis, also, refers *R. hispanicus* Koch, *R. rugosus* Campd., and *R. thyrsiflorus* Fingerh. to *R. Acetosa* L. Jaretsky (1928) suggests that *R. rugosus* is probably a variety of *R. Acetosa*.

<i>R. hispanicus</i> Koch	Jaretsky, 1928
<i>R. montanus</i> Desf.	Ono, 1930a; Takenaka, 1930
<i>R. nivalis</i> Hegetschw.	Kihara and Ono, 1926
<i>R. rugosus</i> Campd.	Jaretsky, 1928
<i>R. thyrsiflorus</i> Fingerh.	Meurman, 1925a, b
<i>R. tuberosus</i> L.	Jaretsky, 1928
CHENOPODIACEAE	
(♀, 2A + 2X ₁ + 2X ₂ ; ♂, 2A + X ₁ + X ₂ + Y)	
<i>Atriplex hymenelytra</i> (Torr.) Wats.	Billings, 1934
ROSACEAE	
(♀, 2A + X + Y; ♂, 2A + 2X)	
<i>Fragaria elatior</i> Ehrh.	
(<i>F. moschata</i> Duchesne)	Kihara, 1926, 1929b, 1930
DIOSCOREACEAE	
(♀, 2A + 2X; ♂, 2A + X)	
<i>Dioscorea sinuata</i> Vell.	Meurman, 1925a, b

TABLE 2

ANGIOSPERMS: RECOGNIZABLE SEX CHROMOSOMES REPORTED ABSENT

Species	References
GARRYACEAE	
<i>Garrya elliptica</i> Dougl.	Meurman, 1930
MYRICACEAE	
<i>Myrica carolinensis</i> Mill.	Stokes, 1937
<i>M. cerifera</i> L.	Stokes, 1937
<i>M. pumila</i> (Michx.) Small	Stokes, 1937
<i>M. Nagi</i> Thunb. (<i>M. rubra</i> S. & Z.)	Sugiura, 1927
LORANTHACEAE	
<i>Arceuthobium Oxycedri</i> (DC.) M.B.	Pisek, 1924
<i>Viscum album</i> L.	Pisek, 1923; Steindl, 1935
POLYGONACEAE	
<i>Rumex Acetosella</i> L.	Meurman, 1925a, b; Kihara, 1925, 1927
CHENOPODIACEAE	
<i>Atriplex Babingtonii</i> Woods.	Wulff, 1937
<i>Spinacia oleracea</i> L.	Strasburger, 1909a, 1910b; Winge, 1923; Tuschnjakowa, 1929; Sinotô, 1929b; Haga, 1935; Lorz, 1937; Araratjan, 1939
AMARANTHACEAE	
<i>Acnida tuberculata</i> Moq.	McWilliams, 1930
CARYOPHYLLACEAE	
<i>Silene Roemerii</i> Frivald.	Schatz (reported by Correns, 1928)
RANUNCULACEAE	
<i>Clematis heracleaefolia</i> DC.	Nakajima, 1937
<i>C. virginiana</i> L.	Lindsay, 1929, 1930
<i>Thalictrum Fendleri</i> Engelm.	Kuhn, 1928, 1930b

MENISPERMACEAE	
<i>Cocculus villosus</i> DC.	Joshi and Rao, 1935
<i>Menispermum canadense</i> L.	Lindsay, 1929, 1930
<i>Tinospora cordifolia</i> Miers.	Joshi and Rao, 1935
SAXIFRAGACEAE	
<i>Ribes alpinum</i> L.	Meurman, 1925a, b
<i>R. orientale</i> Desf.	Meurman, 1928
<i>R. saxatile</i> Pall.	Meurman, 1928
EUPHORBIACEAE	
<i>Mercurialis annua</i> L.	Strasburger, 1909a, 1910b; Malte, 1910; Yampolsky, 1925; Sztajger-waldówna, 1929
<i>M. perennis</i> L.	Sykes, 1909
VITACEAE	
<i>Vitis cinerea</i> Engelm.	Kobel, 1929
<i>V. rupestris</i> Scheele var. <i>metallica</i>	Kobel, 1929
<i>V. tinifera</i> L.	Kobel, 1929
MALVACEAE	
<i>Napaea dioica</i> L.	Bunten, 1929
CARICACEAE	
<i>Carica Papaya</i> L.	Meurman, 1925b; Sugiura, 1927; Lindsay, 1930
ELEAGNACEAE	
<i>Shepherdia canadensis</i> (L.) Nutt.	Cooper, 1932
UMBELLIFERAE	
<i>Trinia hispida</i> Hoffm. (<i>T. Hoffmanni</i> Bieb.)	Araratjan, 1939
CORNACEAE	
<i>Aucuba chinensis</i> Benth.	Kihara and Yamamoto, 1935
<i>A. japonica</i> Thunb.	Sugiura, 1927; Meurman, 1929; Sinotô, 1929b; Yamamoto, 1937
CUCURBITACEAE	
<i>Bryonia dioica</i> Jacq.	Sykes, 1909; Strasburger, 1909a, 1910b; Meurman, 1925b; Lindsay, 1929, 1930
NAJADACEAE	
<i>Najas marina</i> L. (<i>N. major</i> All.)	Winge, 1927a; Takamine, 1927
ALISMACEAE	
<i>Sagittaria montevidensis</i> Cham. & Schlect.	Sykes, 1909
HYDROCHARITACEAE	
<i>Hydrocharis Morsus-ranae</i> L.	Sykes, 1909; Tuschnjakowa, 1929
<i>Vallisneria gigantea</i> Graebn.	Jørgensen, 1927
<i>V. spiralis</i> L.	Winge, 1927a; Jørgensen, 1927
CYPERACEAE	
<i>Carex grillatoria</i> Maxim.	Nakajima, 1937
PALMAE	
<i>Phoenix canariensis</i> Hort.	Beal, 1937
<i>P. dactylifera</i> L.	Beal, 1937
<i>P. sylvestris</i> Roxb.	Beal, 1937
LILIACEAE	
<i>Asparagus officinalis</i> L.	Shoji and Nakamura, 1928; Kamo, 1929; Flory, 1932
<i>Smilax herbacea</i> L.	Elkins, 1914; Lindsay, 1929, 1930
DIOSCOREACEAE	
<i>Dioscorea</i> sp.	Strasburger, 1909a
<i>D. caucasia</i> Lipsky	Meurman, 1925a, b

Allosomes in Secondary Hermaphrodites

Bělař (1925) found in 9 hermaphroditic plants of *Lychnis alba* the allosome complement ($X + Y$) characteristic of ♂♂. These ♀♀ thus fitted the conclusion, based upon experimental work, of Shull, G. and P. Hertwig, and Winge, that the *Lychnis* ♀♀ studied by them, except one reported by Shull, were mutated ♂♂. That secondary ♀♀ may arise by a mutation in the ♀ line, however, is shown by Åkerlund's (1927) finding of a hermaphroditic plant of *L. dioica* with the ♀ complement ($2A + 2X$). Bělař found the ♂ complement also in a thelygenous male, as well as in the gynandromorph described by Correns which bore ♂ flowers in its lower portion, ♀ flowers above.

A hermaphroditic tree of *Populus tremuloides* was found by Erlansson and Hermann (1927) to possess the XY pair characteristic of ♂♂ of the same species.

Allosomes and Heteroploidy

In certain instances hermaphroditism and other sexual conditions unusual for the species appear to be related to the occurrence of atypical chromosome combinations. The species most studied in this connection, almost exclusively by Japanese cytologists, is

Rumex Acetosa. Results obtained by Ono (1928–1935), Ono and Shimotomai (1928), Kihara and Yamamoto (1931), Takenaka (1931, 1937), and Yamamoto (1932–1935, 1938) are summarized by Ono (1935) and Yamamoto (1938). Apart from differences between races in the morphology of individual autosomes, the species presents an extensive array of heteroploid forms whose chromosome numbers range from the typical 14 (♀) or 15 (♂) to 51. The origin of heteroploidy is referred chiefly to the functioning of gametes with unreduced chromosome numbers, and to meiotic irregularities in the resultant polyploid plants.

The relation between chromosome complement and sex in *R. Acetosa* is explained by the workers cited in harmony with the theory of Bridges (1939), originally based upon the genetic behavior of *Drosophila*. In plants which are euploid for the autosomes, sex usually depends upon the ratio between number of A's (autosome complement of 6) and X's. The presence or absence of Y chromosomes (Y_1 and Y_2), or their number if present, seems to have no bearing upon the sex of the plant. If there are two A's or

more to each X, the plant is ♂; *e.g.* (neglecting the Y's): 2 A + X (the ordinary diploid ♂), 3 A + X, 4 A + 2 X. If there is one A to each X, the plant is ♀; *e.g.*, 2 A + 2 X (the ordinary diploid ♀), 3 A + 3 X, 4 A + 4 X, 5 A + 5 X. If the ratio A:X is less than 2:1 and more than 1:1, the plant is in varying degree "intersexual"; *e.g.*, 3 A + 2 X, 4 A + 3 X, 6 A + 4 X. As intersexes are considered plants bearing either bisexual and ♂ flowers (andromonoecious) or bisexual, ♀, and ♂ flowers (trimonoecious). It is concluded that the X chromosome bears a gene or genes tending toward femaleness, and that some of the autosomes carry genes tending toward maleness, the Y chromosomes being indifferent so far as sex is concerned.

Some intersexes with ordinary diploid chromosome complements also occur in nature. These have either 2 A + 2 X (♀ intersexes) or 2 A + X + Y₁ + Y₂ (♂ intersexes). Others have appeared in certain racial crosses. It is agreed that intersexuality in either of these cases is due to changes in some of the autosomes. Takenaka (1937) holds, on the basis of matings involving ♀ and ♂ intersexes, that the changes in autosomes have resulted from irregularities in division. Yamamoto (1938) has obtained diploid intersexes in consequence of X-irradiation of flower buds. He suggests that in these cases male- or female-determining genes were modified.

In aneuploid plants, possessing one or more autosomes or autosomal fragments in addition to 2, 3, or more full sets, the sexual conditions are even more complex. In general, at least 2 extra autosomes are necessary to transform an otherwise ♀ or ♂ plant into an intersex. But there are exceptions; apparently the result depends upon which are the extra autosomes. Yamamoto's (1938) extensive studies of aneuploid forms convince him that genes tending to maleness are located on 3 (and probably on 4) of the autosomes in each set of 6; and that female-tendency genes are present on 2 of the autosomes as well as on the X chromosome.

Lychnis. In this genus, results of changes in chromosome complement seem to be somewhat similar to those manifested in *Rumex Acetosa*. Westergaard (1938), by the use of colchicine, has obtained tetraploid plants of *Lychnis alba*; Warmke and Blakeslee (1939) have reported similar results with a "white-flowered race" of *L. dioica* (possibly *L. alba*). In both cases plants with 4 A + 4 X chromosomes (twice the typical ♀ complement) are ♀; those with

4 A + 2 X + 2 Y (twice the usual ♂ complement) are ♂. By intercrossing these tetraploids, Warmke and Blakeslee obtain the following additional combinations (the "intersexes" of *Rumex* are here represented by ♂♀):

♀: 3 A + 3 X, 4 A - 1 + 3 X.

♂: 3 A + 2 X + Y (1 plant ± ♀), 4 A - 1 + 2 X + Y, 4 A + 3 X + Y (3 ± ♀), 4 A + 1 + 3 X + 2 Y.

♂: 4 A + 1 + 4 X + Y.

The numbers are still small. The fact, however, that one plant with 4 A - 1 + 3 X chromosomes is ♀, whereas 65 with 4 A + 3 X + Y and 3 with 4 A + 1 + 3 X + 2 Y are ♂, suggests that the Y chromosome carries a male-tendency factor, which seems not to be the case in *Rumex*.

Cannabis sativa. Blakeslee (1939) reports that in hemp likewise, doubling the chromosome number of ♀ or of ♂ plants does not affect sex-expression.

Empetrum. A different method of origin of hermaphroditism is suggested by Hagerup's (1927) study of *E. hermaphroditum*. The dioecious *E. nigrum* has 13 chromosome pairs, one of which in the ♂ is unequal (X, Y). *E. hermaphroditum*, with bisexual flowers, has 26 pairs, including 2 X's and 2 Y's—double the complement of the ♂ *E. nigrum*. In meiosis these separate in such a way that each spore receives an X and a Y. On the other hand, Blackburn (1938) has found that an English hermaphroditic *E. nigrum* has but 13 chromosome pairs.

Trichosanthes. *T. cucumeroides* is tetraploid in terms of *T. japonica*, the latter species having 11 chromosome pairs, the former 22. Each (table 1) has a single pair of allosomes.

Hydrilla verticillata. Of this species (Sinotô, 1929b), two forms occur having respectively 16 and 24 chromosomes. In each there is a single XY pair. Sinotô leaves open the question whether the triploid (24-chromosome) form may not be in some degree intersexual.

Salix. In occasional triploid offspring of the cross *S. viminalis* × *S. caprea*, Håkansson (1929, 1938) found no intersexuality. *S. viminalis* is diploid (Blackburn and Harrison, 1924), with one XY pair in the male. Harrison (1926) found *S. lucida*, *S. aurita*, and *S. cinerea* to be tetraploid (with 76 chromosomes) and *S. Andersoniana* to be hexaploid (with 114). In each of these cases,

whether diploid, tetraploid, or hexaploid, the ♂ possesses one XY pair. *S. sachalinensis* (Sinotô, 1929b) has 38 chromosomes, including an unequal pair in the ♂. A form of the same species from Hokkaido was found to have a larger number, apparently about 48, with but one XY pair.

Aucuba chinensis with 16 chromosomes and *A. japonica* with 32 present another case of polyploidy (see references in table 2; also Meurman, 1931). Since, however, no allosomes are recognized in either species, no light is thrown upon the question of the relation of allosomes to polyploidy. The case of *Vallisneria spiralis* with 20, and *V. gigantea* with 40 chromosomes, is similar (Jørgensen, 1927). So is that of *Spinacia oleracea* and the tetraploid plants obtained from it by colchicine treatment (Blakeslee, 1939), except that in this instance some ♂♂ appear in addition to ♀♀ and ♂♂.

Allosomes and Hybridity

Meurman (1925b) considers *Populus Simonii* a hybrid because of the variable behavior of the chromosomes during meiosis. An unequal chromosome pair is present in the ♂ (table 1); presumably, the plant being a hybrid, the two members of this pair must have been derived from the respective parental forms.

Hybrids between various species of *Salix*, including *S. aurita*, *S. Caprea*, *S. purpurea*, and *S. viminalis* which are reported to possess sex chromosomes, have been studied by Blackburn and Harrison (1924) and Håkansson (1929, 1933, 1938). In no instance, apparently, were sex chromosomes recognized. Håkansson, indeed, like Meurman (table 2), recognized no such bodies in pure *S. Caprea*.

In hybrids between *Lychnis alba* and *L. dioica*, Blackburn (1924, 1929) found an XY pair behaving exactly as does the corresponding pair in each of the parent species.

Yamamoto (1935) describes reciprocal crosses between *Rumex Acetosa* and *R. montanus*, which have similar chromosome complements. The hybrid offspring in each case resembled the parent species in their strict dioecism, and in the behavior of the tripartite allosome complex in the ♂♂.

Origin of Sex Chromosomes

Blackburn (1928) found in various species of *Silene* 12 pairs of chromosomes. In some, as *S. nutans*, the chromosomes are of ap-

proximately equal size; in others, as *S. viridella*, one pair is noticeably larger than any of the others. She suggests (1929) that a cross between two such species would result in a hybrid with an unequal pair. If this were back-crossed to the parent with a large pair, the next generation would consist of some individuals with an unequal pair and others with an equal large pair. This would correspond, as regards chromosome sizes, to the condition in ♂♂ and ♀♀ respectively of the dioecious *S. Otitus*, and of the related dioecious species of *Lychnis*.

Winge (1932, 1938) has proposed a different hypothesis, based largely upon his own work with the fish *Lebistes reticulatus*. In some of these experiments, in consequence of crossing over, the XY pair lost its function in sex-determination, which function was assumed by another pair of chromosomes. Winge assumes that all chromosomes bear numerous "relatively weak sex genes." Crossing over may result in one member of a pair bearing predominantly male-tendency genes, the other member predominantly those with a ♀ tendency. These particular chromosomes then become sex-determining.

As to the tripartite complex of *Rumex Acetosa* and related dioecious species, Jaretsky (1928) suggests that it has arisen by fusions between members of the hexapartite ring or chain often seen in *R. Acetosella*. Fusions among supposedly homologous autosomes would account for a reduction of the 36 of *Acetosella* (apart from the ring) to the 12 of *Acetosa*. Ono (1935) considers it more likely that *R. Acetosa* is descended from a hermaphroditic species which, like some still extant, possessed 16 chromosomes. Reciprocal translocations between members of two pairs, resulting in new groupings of sex-tendency genes, would account for the tripartite chain of allosomes, leaving the remaining 12 (6 pairs) to function as the autosomes of *R. Acetosa*.

Differences between Microgametophytes

Further evidence that ♂ plants of dioecious species are heterogametic for sex-tendency factors is furnished by Correns' (1928) comparison of the effects of placing very small and very large numbers of pollen grains on the stigmas of ♀ plants. Earlier studies had shown the sex ratio in dioecious species (number of ♂♂ to number of ♀♀) to be very variable in nature as well as under cul-

tural conditions (see especially Strasburger, 1900). In general, ♀♀ exceed ♂♂ in number, often very greatly. In *Lychnis dioica*, *L. alba*, and *Rumex Acetosa*, Correns found abundant pollination to result in a larger proportion of ♀♀ among the offspring than did scanty pollination. In the latter case the proportion of ♂♂ sometimes approached, though it did not in general reach, 50% of the total progeny. He concluded that, in the competition between pollen tubes carrying "female-determining" and those carrying "male-determining" gametes, the former are favored either by more rapid germination of the pollen grains or by a more rapid growth of the tubes, or in both ways. If competition is reduced by the presence of but few pollen grains, the chances for the functioning of the two types of ♂ gametes approach equality. The treatment of dry pollen grains of *Lychnis* with alcohol vapor, or the aging of pollen, increased the proportion of ♂ offspring, presumably because of a greater mortality of the female-determining grains. The age of eggs, on the contrary, had no influence on the sex ratio, as was to be expected if the ♀ is homogametic.

Tischler (1925) found that of *Lychnis* pollen subjected at the time of germination to alcohol vapor, a higher proportion of the smaller grains and a lower proportion of the larger grains germinated than in the case of pollen placed under similar conditions save for the absence of alcohol. These, taken with Correns' results, suggest that female-determining are on the average larger than male-determining pollen grains. Measurements indicated that the ratio of surface area of the vegetative nucleus to the volume of the grain is higher for the smaller than for the larger grains.

As regards the effect of the age of pollen upon the sex of the offspring, reports of experiments with *Cannabis sativa* are conflicting. According to Cieselski (1911), the use of fresh pollen resulted in an exclusively or almost exclusively ♂ progeny, that of pollen 12 hours old in a wholly ♀ progeny. Lilienfeld (1921) found that the use of fresh pollen resulted in a predominance (about 62%) of female offspring; pollen 12 hours old gave essentially similar proportions; but when pollen 30–36 hours old was applied, only about 57% of the offspring were ♀♀. Although the difference is doubtfully significant, his results so far as they go agree with Correns' (with *Lychnis*) that the aging of pollen tends to an increase in the proportion of ♂ offspring. On the other

hand, Riede (1925), giving no details, reported that aging of pollen reduces the proportion of ♂♂. He found, like Correns, that sparse pollination, diminishing the competition between pollen tubes, increases the proportion of ♂♂. Sinotô (1929b) determined that the distribution of pollen grains of *Cannabis* according to size presents a bimodal curve.

In *Humulus japonicus*, Kihara and Hirayoshi (1932) found that abundant pollination results in an excess of ♀ offspring.

Santos (1924) observed in *Elodea canadensis* and *E. gigantea* that of the 4 pollen grains of a tetrad (which remain adherent in *Elodea*) 2 are smaller than the other 2.

It is of interest that in each of the species employed in the experiments above mentioned, the pollen grains are now known to be of two sorts in terms of their chromosome complements.

Sex-linked Inheritance

This phenomenon involves such a distribution of particular non-sexual characters as to indicate that they are determined by genes borne on a sex chromosome. Many sex-linked characters are known in animals, connected chiefly with genes on the X chromosome. That only a few such characters have been recognized in angiosperms may well be because so little detailed genetic work has been done with dioecious species.

The first clear case of sex-linkage in plants was briefly reported by Baur (1912) and more fully studied by Shull (1914). A ♂ plant of *Lychnis alba* appeared in Baur's cultures, differing from typical members of the species particularly in its very narrow leaves. This plant, mated with a typical ♀, produced an F₁ progeny all broad-leaved. Matings between members of the F₁ generation resulted in F₂ progenies of broad-leaved ♀♀, broad-leaved ♂♂, and narrow-leaved ♂♂. No narrow-leaved ♀♀ appeared. This behavior parallels that characterizing sex-linked characters in animals with an X and a Y chromosome in the ♂, as for example in crosses of a red-eyed ♀ × a white-eyed ♂ in *Drosophila*. Further crosses involving descendants of the original narrow-leaved ♂ *Lychnis* gave results of the nature expected (with the exception of one unexpected narrow-leaved ♀ "mutant") on the assumption of a gene determining broad leaves, or its recessive allele for narrow leaves, borne on the X chromosome, no corres-

ponding gene being borne on the Y. In certain families the expected ♀♀ were nearly or quite absent—the condition of arrhenogeny already mentioned. One ♂ (broad-leaved) was mated with a ♀ heterozygous for the narrow-leaf gene. The offspring included broad-leaved ♀♀, broad-leaved ♂♂, and narrow-leaved ♂♂. This result was the same, substituting ♂♂ for ♂♂, as would have been expected had the father been a ♂. It is further confirmation of Shull's previous conclusion that the group of ♂♂ to which this particular parent belonged were mutated ♂♂. However, a broad-leaved ♂ from a different source, mated with a heterozygous ♀, produced only broad-leaved ♀♀. This latter hermaphrodite appears to have been a mutated ♀, like the one studied cytologically by Åkerlund (1927).

Winge (1927b) explains Shull's cases of arrhenogeny by a lethal effect upon pollen grains of the narrow-leaf gene borne on the X chromosome. In a similar category comes Correns' (1928) suggestion that thelygeny is due to a lethal or sublethal gene on the Y chromosome.

Winge (1927b) found, in an F_2 progeny from a cross of *Lychnis dioica* × *L. alba*, a group of ♂♂ which were yellow (*chlorina*, later called *aurea*) instead of the typical green. Offspring of crosses in which the *aurea* ♂♂ functioned included green ♀♀, green ♂♂, a few green ♂♂, and *aurea* ♂♂. Since all the *aurea* plants in various families were ♂♂, although in certain cases homozygous *aurea* ♀♀ were expected, it was thought that the *aurea* character is due to a recessive gene borne on the Y chromosome.

Later, on the basis of more extended experiments, Winge (1931a, b) concluded that the *aurea* factor is borne on the X chromosome, and that it is lethal to ♀♀ which are homozygous for this recessive factor. Such ♀♀ would be yellow if they appeared. The situation is complicated by the existence of an autosomal gene *A* which inhibits the effect of the *aurea* gene. Hence a ♀ with two recessive *aurea* genes may exist if it has one or two *A* genes, but because of the presence of the latter it is green. Such a ♀, however, if it is heterozygous for the *A* gene (having *A* and *a*) and has one or two *aurea* genes, may give rise, as Winge's experiments showed, to *aurea* ♂♂.

Three cases of sex-linked genes appearing among the offspring of crosses between *Lychnis dioica* and *L. alba* have been reported

by Winge (1931*b*). One, borne by the Y chromosome, inhibits the appearance of variegation. Consequently all variegated plants are ♀♀; the ♂♂, possessing a Y chromosome, are never, or only very slightly, variegated. Another case is that of an "abnormal" character, affecting more or less the whole plant. This character appears, in certain families, only in ♀♀; in other families, only in ♂♂. Its distribution is explained by a recessive gene borne on the X or Y chromosome or on both. Its effect is inhibited by certain autosomal genes, as well as by the sex-linked *aurea* gene. The third case is that of the lethal X-linked gene previously mentioned, present in some ♀♀ and preventing the appearance of ♀♀ in their progeny.

Imai (1938) finds two mutant characters, yellowish-green and yellow-mottled, in plants of hemp resulting from the use of X-rayed pollen. In each case the new character appeared only in ♂♂. He considers that both genes involved are located on the X chromosome.

Hofmeyr (1938*a, b*) reports that in *Carica Papaya* a gene pair affecting flower color (yellow or white) shows an apparent linkage with the genes differential for sex-expression.

EXPERIMENTAL STUDIES OF PLANTS OF OTHER CATEGORIES

The problem of sex-inheritance in those angiosperms (the great majority) which fall within other categories than dioecism has, with one exception, been relatively little studied. The exception is, of course, the monoecious *Zea Mays*. Otherwise, results are scattered, although all told a considerable number of mutations affecting sex-expression have been observed. One large class includes those which result in "doubling" of flowers, in so far as these involve a transformation of stamens or of both stamens and pistils into sterile petaloid structures. No attempt will be made to list mutations of this type.

Gynodioecious Species

It has been seen that in many species classed as dioecious, not only may some ♀ flowers appear on ♂ plants and *vice versa*, but bisexual flowers also may be borne on plants of either sex. Most commonly, it appears, it is the ♂ plants which produce the exceptional flowers. This fact may well be related to the heterozygosity

of the ♂ in most dioecious species for a determinative sex-tendency factor.

Considered phenotypically, the appearance of bisexual flowers on ♂ plants is transitional to gynodioecism—the occurrence of both ♀♀ and ♂♂ within a species. Likewise, the appearance of bisexual flowers on a ♀ plant grades into androdioecism. In either case, if all or most of the flowers of a plant become bisexual, the plant is a secondary ♂.

That the occurrence of secondary ♂♂ may lead to gyno- or androdioecism is illustrated by the appearance already cited of ♂♂ among cultivated grapes and strawberries derived from dioecious species. Selection has led to the perpetuation of both hermaphroditic and ♀ strains as distinct varieties. In effect, mutation and selection within dioecious species have led to gynodioecism.

Such an occurrence, however, seems to be a case of backward evolution. There is little reason to doubt that present gyno- and androdioecious species have been in the main derived from the primitive hermaphroditic condition by way of mutations which resulted in male or female sterility. These mutations seem to tend to occur more frequently in the direction of male than in that of female sterility. Male sterility, should it become a fixed condition in some individuals of a previously hermaphroditic species, would result in gynodioecism. It is probably not a coincidence that gynodioecism is more widespread than androdioecism. Either condition may be considered a step transitional between hermaphroditism and dioecism.

Several studies have been made of species which are classed as gynodioecious, although, as in most dioecious species, variations from the typical condition occur. Apparently there are no similar studies on forms in which androdioecism is the settled condition.

Among the most clear-cut results are those of Correns (1907, 1916, 1928) with *Satureia hortensis*, *Cirsium oleraceum*, and (on a limited scale) *C. acaule*. Plants of these species are of two kinds: one strictly ♀, the other more or less hermaphroditic but bearing, in addition to bisexual flowers, a varying proportion which are structurally or (with abortive anthers) functionally ♀. Those classed as ♂♂ apparently include genetically different strains. The ♀♀ constitute a uniform class. Hermaphrodites, selfed, produced, save for occasional ♀♀, offspring like the parents. Females × ♂♂

yielded a progeny wholly ♀. Females pollinated from other species of various types (*C. acaule*, *C. palustre*, *C. canum*, and *C. Erisithales*) likewise gave only ♀♀. Correns at one time (1907) considered the latter results parallel to those of the cross of *Bryonia dioica* × *B. alba*, the ♀ tendency carried by the eggs being dominant to the hermaphroditic tendency carried by the ♂ gametes. Later (1928), he was inclined to accept an explanation suggested by Wettstein (1924), similar to one offered by Chittenden and Pellew (1927) for the case of flax to be mentioned later: namely, that the cytoplasm of ♀ plants, transmitted through the eggs, modifies the effect of genes borne by some or all of the ♂ gametes to the extent that in the offspring the development of stamens is checked or inhibited.

The behavior of other gynodioecious species is less simple. In *Silene inflata* and *S. dichotoma* some plants are strictly ♀, some strictly hermaphroditic, some bear a variable proportion of ♀ in addition to bisexual flowers. The latter two classes behave alike in inheritance and are classed by Correns (1906*b*) as "± hermaphroditic." Females × ♂♂ yield mostly ♀♀ and a small proportion of ♂♂. Hermaphrodites selfed produce chiefly ♂♂ plus a few ♀♀. Correns' (1916, 1928) results with *Cirsium palustre* are similar, except that ♀♀ × ♂♂ produce a larger proportion of ♂♂, and that ♂♂ selfed produce variable, sometimes large, proportions of ♀ offspring. The experiments with *C. Velenovskyi* were limited and not conclusive.

Plantago lanceolata (Correns, 1928) gives even more confusing results. The numerous types which can be distinguished in this species are combined by Correns into five groups: ♀♀, ♂♂, and 3 intermediate classes varying in the proportion of functionally ♀ flowers (having imperfect anthers). The same ♀ mated with different ♂♂ or intermediates gave progenies with very different proportions of the various classes. Likewise, different ♀♀ pollinated from the same ± hermaphroditic plant produced differing progenies. The conclusion, from this case and those previously mentioned, was that the gametes of each sexual form tend to produce offspring like the parent; and that the tendency of the phylogenetically younger form (unisexual, in this case ♀) is dominant to that of the older form. It is evidently necessary also, especially to account for the conditions in *Plantago*, to assume either varying strengths

(valencies) of the respective tendencies, or a considerable number of genic differences between the numerous strains within a species.

Majorana hortensis (sweet marjoram), studied by Appl (1932), likewise presents, in addition to ♀♀ and ♂♂, intermediates with varying proportions of ♀ and bisexual flowers. Females × ♂♂ give approximately 69% ♀♀, 31% ♂♂. Hermaphrodites selfed or intercrossed produce a majority of ♂♂, a minority (24.5% and 49.65% in different cases) of ♀♀. Appl assumes an allosome complement for ♀♀ of XX; for intermediates, XY; for strict ♂♂, YY. Rare forms are explained by non-disjunction, giving rise to such combinations as XXY and XYY.

Among plants of *Polemonium coeruleum* Ostenfeld (1923) finds likewise 3 types. His "intermediates," however, are ♀♀ with an occasional stamen more than ordinarily developed but usually producing no viable pollen. Females × ♂♂ yielded ♀♀ and a few (average 9.5%) ♂♂. "True" ♂♂ selfed produced ♂♂ plus about one % ♀♀. However, F₁ ♂♂ from ♀ × ♂, when selfed, yielded 2 ♀♀ : 1 ♂. An intermediate × a true ♂ gave a similar progeny. Finally, ♂♂ of a micropetalous strain, selfed, produced ♂♂ plus 28% ♀♀.

The complex situation in *Campanula carpatica*, studied by Pellew (1917), is not yet fully analyzed. Besides ♀♀ and ♂♂, 4 intermediate classes are recognized: 2 functionally ♀, 2 functionally hermaphroditic. Matings in which ♂♂ were intercrossed in some cases produced families all fully hermaphroditic; in other instances, various or all 6 recognized classes were represented, but with ♂♂ in the majority. Females × ♂♂ yielded similarly divergent families but with a majority of ♀♀. One ♂ behaved peculiarly; used as a ♂ parent, mated with ♀♀, the offspring were all ♀♀; as a ♀ with other ♂♂, the offspring were all ♂♂; but as a ♂ with other ♂♂, the offspring were either all ♂♂, or mixed with ♀♀ the more numerous.

Raunkiaer (1906) sowed seeds from ♀♀ (presumably pollinated from ♂♂) and from ♂♂ (presumably selfed or intercrossed), of *Thymus vulgaris* and *Knautia arvensis*. Of the former species, seeds from ♀♀ gave rise to 40 ♀♀, 2 ♂♂; seeds from ♂♂ to one third ♂♂, two thirds ♀♀. Seeds from ♀♀ of *Knautia* gave rise to 82% ♀♀, 18% ♂♂; of the seeds from ♂♂, 94% produced ♂♂ (including some intermediates), 6% ♀♀. Similar sowings by Lavialle and Jaeger (1934) of seeds of *Knautia* resulted not very differently. Seeds from ♀♀ produced 76% ♀♀, 24% ♂♂; seeds from ♂♂, exclusively ♂♂.

Species Displaying Monoecism and Related Conditions

Dimorphotheca pluvialis, previously classed as gynomonoecious, was found by Correns (1906a) to be really trimonoecious. The ray flowers are ♀, most of the disc flowers hermaphroditic, the innermost disc flowers ♂. Seeds from ray flowers and from hermaphroditic disc flowers gave progenies similar as to the distribution within each group of the numbers of ray flowers. Correns concluded that the embryos in the seeds of the two classes are genotypically alike despite differences in sex-expression between the flowers in which they were formed.

Cucurbitaceae. A cross of *Cucumis flexuosus* (monoecious) × *C. chinensis* (hermaphroditic) was made by Pangalo (1936). All the F_1 generation were monoecious. The F_2 plants were of 13 types, distinguished by the sex of their flowers and by the position of the ovaries. Among them were ♀♀, ♂♂, and ♂♀. Pangalo concludes that sex-expression in *Cucumis* is determined by at least 10 genes.

In *Cucumis Melo* (melon), *C. sativus* (cucumber), and *Citrullus vulgaris* (watermelon), both monoecious and andromonoecious varieties occur. Rosa (1928) found that andromonoecious plants of melon, selfed, produced a wholly andromonoecious progeny. Reciprocal crosses between andromonoecious and monoecious varieties yielded monoecious offspring. F_1 plants, selfed or intercrossed, gave an F_2 ratio of 3 monoecious : one andromonoecious. F_1 (monoecious) plants, back-crossed to the andromonoecious parent, produced approximately equal numbers of monoecious and andromonoecious offspring. Like results followed similar crosses between varieties of cucumber and of watermelon. Thus the difference between andromonoecism and monoecism behaved as though determined by a single pair of genes, monoecism being dominant. Rosa suggested that the monoecious condition may have arisen from andromonoecism by a dominant mutation which inhibited the development of stamens in previously bisexual flowers.

Poole and Grimball (1939) found 5 ♂♂ among plants of *Cucumis Melo* introduced from China. These were crossed with andromonoecious and monoecious varieties. Andromonoecious × ♂ gave an andromonoecious progeny. The F_1 plants selfed gave an F_2 progeny of 3 andromonoecious : one ♀. Hence it appears that andromonoecism differs from hermaphroditism in one gene pair, hermaphroditism being recessive. Offspring of monoecious × her-

maphrodite were monoecious; F_1 plants, selfed or intercrossed, gave an F_2 generation in the approximate ratio of 9 monoecious : 3 andromonoecious : 3 of a varied group (gynomonoecious, ♀, and trimonoecious) : one ♂. The members of the variable third group are considered to be genetically gynomonoecious but easily modifiable by environmental conditions. This interpretation was supported by a back-cross of the monoecious F_1 plants to the hermaphroditic parent race, which yielded monoecious, andromonoecious, gynomonoecious, and hermaphroditic plants in approximately equal numbers. It is concluded that ♂♂ differ from monoecious plants by two recessive genes. A plant heterozygous for one pair of genes is andromonoecious; one heterozygous for the other pair is gynomonoecious.

Ambrosia elatior (*A. artemisiifolia* L.). In this species, studied by K. L. Jones (1936), three types of plants occur: monoecious, the most frequent form; intermediate, chiefly monoecious but with varying distribution of ♀ and ♂ flowers and with occasional intersexual structures; and ♀. Jones' results indicate that the sex of offspring depends entirely upon the genetic constitution of the mother plant. One class of monoecious individuals pollinated from whatever source produces a wholly monoecious progeny (with very rare intermediate exceptions). A second class, phenotypically indistinguishable from the first, produces offspring of all 3 types; the proportion varies greatly in different families, but on the average roughly 60% are monoecious, 30% intermediate, 10% ♀. Intermediate or ♀ plants likewise produce offspring of all 3 classes; those from intermediate parents average about 35% monoecious, 40+ % intermediate, and 20+ % ♀; those from ♀ parents, about 17% monoecious, 40+ % intermediate, and 40+ % ♀. Jones concludes that many genes, located in several chromosomes, are concerned in sex-expression; the first-mentioned class of monoecious individuals behaves as though homozygous for controlling genes; and all functional ♂ gametes are genotypically alike.

Zea Mays. Of all plant species, this has been most extensively studied genetically. Maize, in its numerous cultivated varieties, is regularly monoecious; but mutations affecting sex-expression are not infrequent. Of the mutant sex-influencing genes which have been recognized and whose genetic behavior has been studied, those listed below are included, and their effects are described, in the summary by Emerson, Beadle, and Fraser (1935):

- Resulting in complete or partial female sterility:
 - Anther ear; 2 genes, one on chromosome 1.
 - Barren stalk; 2 genes, on chromosomes 2 and 3 respectively.
 - Lethal ovule; chromosome 4.
 - Silkless; chromosome 2.
- Resulting in complete or partial male sterility:
 - Antherless.
 - Pollen-lethal; probably on chromosome 5.
 - Male-sterile; 20 genes; one each on chromosomes 1, 3, 5, 6, 8; three on chromosome 9.
 - Tassel seed; 5 genes; one each on chromosomes 1, 2, 3, 4.
- Tending toward both female and male sterility:
 - Asynaptic; chromosome 1.
 - Barren sterile.
 - Cuzcoid.
 - Polymitotic; chromosome 6.
 - Sticky chromosome; chromosome 4.
- Affecting the nature or form of the inflorescence:
 - Hermaphroditic flowers.
 - Development of secondary pistillate florets; two genes.
 - Ramosa ear; two genes, on chromosomes 3 and 7 respectively.
 - Teopod; chromosome 7.

In addition, various genes, such as those for "dwarf" and "pigmy," affect the constitution of the whole plant as well as directly or indirectly the production or functioning of stamens and pistils.

It is evident that there is an indefinitely large number of genes, distributed through at least 9 of the 10 chromosomes, whose presence in the "normal" (usual) condition is essential to the sex-expression typical of the species as it exists at present. With 2 exceptions, tassel seed-3 and tassel seed-5, all the mutant genes above listed are recessive; that is, the genes determining the present typical condition of the species are in general dominant.

Particularly to be noted are the numerous male-sterile genes, whose effect (when homozygous) is shown in varying degrees of incomplete development of anthers or in degeneration of microspores after their formation; in at least one instance a small amount of normal-appearing pollen is produced.

A genetically different type of male sterility, in which microspores are regularly formed but soon degenerate, appeared in a plant from a Peruvian source studied by Rhoades (1933). This plant, being female-fertile, could be pollinated from normally monoecious plants. Breeding experiments indicated that the male-sterile tendency is inherited through the eggs. Among male-sterile offspring, some produced small amounts of good pollen. The close-pollination of such plants, and their pollination from normally fer-

tile plants, gave similar results, showing that the male-sterile tendency is not inherited through male gametes. Other tests showed no relation of male sterility to any of the 10 linkage groups. Rhoades concludes that this is another case of an influence of the egg cytoplasm upon sex-expression.

Hermaphroditic Species

Many of the mutations that have been observed in species of this category, tending toward female or male sterility, are, as previously noted, phenotypically similar to some of those which must be supposed to have given rise to the intermediate conditions, such as monoecism, and ultimately in various lines to dioecism. In hermaphroditic species, as in some of those previously mentioned, a frequent class of mutations consists of those leading to "double-ness," stamens or carpels or both being replaced by petaloid structures.

Male Sterility. Perhaps next most common among sex-influencing mutations in hermaphroditic species are those which, like many previously mentioned in maize, are classed as "male-sterile" or "pollen-sterile," leading toward or to functional femaleness. The effects of such mutations are variable in degree. They may involve the degeneration of pollen grains after their formation; degeneration may occur at the period of meiosis, often leading to "contabescence" of anthers; or development may cease at an earlier period, the result being an abortion of anthers or of stamens, or a failure of stamens to develop at all.

An early observation of a male-sterile mutant was reported by Bateson, Saunders, and Punnett (1908) in a sweet pea. Male sterility in crosses between species of shepherd's purse (*Capsella*) was found by Shull (1927) to be simply recessive to male fertility. Several similar instances cited by Correns (1928) will not be repeated here. Recent reports are by Karper and Stephens (1936) and Stephens (1937) of a recessive male-sterile ("antherless") form of sorghum; by Sterling Emerson (1938) of a recessive male-sterile gene in *Oenothera organensis*; and by Lesley and Lesley (1939) of male sterility in the tomato, influenced by at least two recessive genes. Jones and Emsweller (1937) describe a completely male-sterile plant of Indian Red onion. Krantz, Becker, and Fineman (1939) adduce evidence that pollen sterility in the potato is inherited in simple Mendelian fashion.

Correns (1928) observed strains of *Silene Armeria*, *S. dichotoma*, and *S. mellifera* in which both stamens and petals were replaced by carpels. This aberrant condition in *S. Armeria* proved recessive to typical hermaphroditism.

Forms of the wallflower (*Cheiranthus cheiri*) have long been known with reduced petals and with stamens replaced by carpels. This condition also was found by F. J. Chittenden (1914) and Sirks (1924) to be recessive. Chittenden mentions strains of *Papaver somniferum*, *P. orientale*, and *Polemonium coeruleum* some or all of whose stamens are transformed into carpels.

An extreme case of male sterility is that of *Hieracium excellens*, all whose plants were found by Ostenfeld (1906) to be ♀ in the sense that no viable pollen is formed. Related species are hermaphroditic, at least structurally, although parthenogenesis is known to be frequent in the genus. Some of the eggs of *H. excellens*, being diploid, are parthenogenetic; this fact explains the persistence of the species. Some eggs, however, are haploid and will not develop without fertilization. Fertilized by ♂ gametes from hermaphroditic species, the eggs produced a limited number of ♀♀ and ♂♂.

Male sterility appeared in one fourth of the members of the F₂ generation of a cross made by Bateson and Gairdner (1921) between procumbent and tall races of flax (*Linum usitatissimum*). Both parent races are hermaphroditic. This and later work reported by Gairdner (1929) showed that male-sterile × tall plants produce male-steriles; whereas, male-sterile × procumbent plants yield ♂♂. Male-steriles × F₁ ♂♂ (from tall × procumbent) produce ♂♂ and male-steriles in equal number; but male-steriles × F₂ ♂♂ yield, in different cases, either ♂♂ or male-steriles or equal numbers of the two classes. Gairdner adopts the explanation suggested by Chittenden and Pellew (1927), that the tall race carries a pair of recessive genes for male sterility which can function only in the cytoplasm of the procumbent strain. When a combination is obtained of procumbent cytoplasm with two male-sterile genes, the plant so endowed is phenotypically male-sterile. All other combinations of cytoplasm and genes result in hermaphroditism. According to Sansome and Philp (1932), a similar behavior to that in *Linum* is manifested in a cross of *Geranium Endressi* × *G. striatum*.

In certain of East's (1932) crosses involving *Nicotiana Langsdorfii* and *N. Sanderae*, plants containing only or preponderantly *Langsdorfii* cytoplasm and homozygous for certain self-sterility factors (affecting pollen-tube growth) were male-sterile. In *Sanderae* cytoplasm, male sterility did not appear. East adopts an explanation of cytoplasmic influence on genic expression similar to that accepted by Gairdner for flax.

Ranunculus acris, as found in the Old World and introduced into the New, includes a variety of forms some of which depart from the typical hermaphroditic condition. Sorokin (1927) found a wide range of chromosome numbers within the species, although the chromosomal differences were not always correlated with morphological distinctions. She discovered ♀♀, having small flowers with abortive stamens, growing with ♂♂ and intermediate forms near Leningrad. The "normal" plants had 12 chromosomes; the ♀♀ were triploid, with 18. Of the progeny of a ♀ × a typical ♂, about one fourth were ♀♀, a few ♂♂, the remainder variously intermediate. Sorokin concludes that femaleness is an indication of unbalanced types of changes which have occurred in the chromosome complement.

Marsden-Jones and Turrill (1929, 1935) distinguished 6 classes in British plants of *R. acris*: ♂♂, ♀♀, ♂♂, neuters, and two sorts intermediate between ♂ and ♀ with stamens reduced in different degrees but producing some viable pollen. Members of all these classes (Whyte, 1929; Larter, 1932) have the same chromosome number (14). Marsden-Jones and Turrill concluded that the stoppage of processes leading to pollen-formation in ♀♀ is associated with a coincidence in time of the meiotic divisions in macro- and microspore mother cells, whereas in ♂♂ anther-development precedes by some time ovule-development. In the intermediate forms the two processes are closer together in point of time but not synchronous; hence a greater or smaller amount of pollen is formed. Matings of ♂♂ × ♂♂ showed maleness to be recessive. The authors adopt the factorial scheme used by Crane and Lawrence (1931) for *Rubus* (see below) as approximately fitting their results.

Of the extensive literature dealing with differences between reciprocal hybrids within the genus *Epilobium* when one of the parents is *E. hirsutum* or *E. parviflorum*, only the discussions of

Lehmann and Schwemmle (1927) and of Michaelis (1931, 1933) will be cited. The paper last mentioned includes references to other work. The offspring of *E. roseum* \times *hirsutum* have well-developed flowers and are fairly fertile. Those of the reciprocal cross have reduced petals, anthers, and pistils, and are almost completely male- and female-sterile. Hybrids of *E. luteum* \times *hirsutum* are fertile; those of *E. hirsutum* \times *luteum* are male-sterile. Other differences between reciprocals affect the vegetative organs. Numerous crosses and back-crosses have shown that if *luteum* (with varying proportions of *hirsutum*) chromosomes are present in *hirsutum* cytoplasm, the result is male- (and commonly a large proportion of female-) sterility. When the *luteum* \times *hirsutum* hybrid was back-crossed repeatedly to the *hirsutum* parent, the proportion of *hirsutum* chromosomes thus being increased, male sterility appeared. After five back-crosses, it was assumed by Michaelis that the chromosomes present were entirely or almost entirely derived from the *hirsutum* parent, the cytoplasm being still of *luteum* origin. The plants now displayed chiefly *hirsutum* characters, but most of them were male-sterile, rarely producing small amounts of pollen. Some plants of the same generation produced larger amounts of pollen. It is agreed by those who have discussed these and similar phenomena in *Epilobium* hybrids that the character of the cytoplasm plays a genetic rôle; the conflicting hypotheses as to the nature of this rôle are fully discussed by East (1934).

Oehlkers (1938) reports remarkable results as to sexual conditions in certain crosses between species of *Streptocarpus*. All the species studied are regularly hermaphroditic. In a cross of *S. Rexii* \times *Wendlandii*, the offspring were hermaphroditic but their ovules were sparingly fertile. The reciprocal cross resulted in a complete suppression of anthers (except for very small anthers in one plant). Differences in other floral characters also appeared between the reciprocal crosses. Oehlkers adopts the interpretation of a cytoplasmic influence suggested for other more or less similar cases by Wettstein (1924) and Chittenden and Pellew (1927). In the cytoplasm of *S. Rexii*, the effect of sex-influencing genes of *S. Wendlandii* is somewhat modified in the direction of maleness. In *Wendlandii* cytoplasm, the action of *Rexii* genes is so modified as to result in complete femaleness. The hypothesis is supported by the results of back-crosses and of matings between the F_1

hybrids. When, in consequence of such matings, two *Rexii* genoms were brought into *Wendlandii* cytoplasm, the stamens were modified into more or less carpel-like structures. In extreme cases, stamens were fully transformed into open carpels bearing exposed ovules. In the offspring of crosses of *S. Comptonii* × *Rexii* and *S. Wendlandii* × *grandis*, anthers were absent; in the former, the reduced stamens bore ovules; in the latter, stamens were often absent.

Female Sterility. In hermaphroditic species as in those of some other sexual classes, mutations in this direction appear to be much less frequent than are those tending to male sterility. Two cases are reported of "*calycanthema*" forms, in which the calyx becomes petaloid. Correns (1905, 1928) found the pistils in certain *calycanthema* strains of *Campanula Medium*, although fully developed, to be nearly or quite sterile. Complete sterility of pistils is described by Ikeno (1923) in a *calycanthema* form of *Rhododendron indicum* var. *Kaempferi*. Both authors find the ♂ (female-sterile) dominant to the typical hermaphroditic condition.

Witte (1919) found that certain plants of *Phleum pratense* (timothy grass) were almost completely female-sterile, with abortive pistils. Limited breeding results indicated that female sterility is recessive.

Rainio (1937) made interracial crosses within the hermaphroditic species *Geranium pratense*. When a particular light-green race furnished the pistil parent, the offspring possessed "intersexual" pistils showing transitions toward the staminate structure, although still functional. Certain back-crosses produced progenies either wholly intersexual or half intersexual, half typical.

In Correns' (1928) cross of *Geum urbanum* × *rivale* (both hermaphroditic), among the F_1 offspring appeared varying combinations of bisexual and ♂ flowers ranging from hermaphroditic plants with rare ♂ flowers to plants wholly ♂. The results of matings among plants of these new types were not clear-cut.

A different type of mutation which may be said to represent a tendency toward maleness although female-sterility is not involved, consists in the replacement of petals by stamens. For over a century, forms of the shepherd's purse (*Capsella Bursa-pastoris*) have been observed in which such a replacement involves some or all of the 4 petals. Crosses made by Dahlgren (1919) between a race

with 10 stamens instead of 6 and no petals, and representatives of 3 species with typical flowers, showed the "decandrous" form dominant to the typical. Shull's (1929) results with a like form agreed with Dahlgren's.

A similar mutant condition has been long known also in the foxglove (*Digitalis purpurea*). In this case the 3 lower corolla lobes, and occasionally the 2 upper lobes, are partly or entirely transformed into stamens. Saunders (1911) and Shull (1912) found the modified condition to be recessive.

Female and Male Sterility. Among the mutants that appeared in Baur's (1924) cultures of *Antirrhinum*, the following, each recessive to the typical form, are characterized among other things by modifications of the sex organs: *globosa*, with stamens reduced or (usually) absent, very rarely producing pollen; *nicotianoides*, anthers nearly always abnormal and sterile; *ericoides*, anthers always sterile; *globifera*, anthers absent; *cornuta*, ovary often reduced and more or less sterile; *sterilis*, flowers replaced by clusters of sepal-like leaves.

Nagai (1926) has described six mutant forms occurring in families derived from a cross between two varieties of rice (*Oryza sativa*). In one of these ("staminoidal sterile") the stigma is transformed into anthers, hence the plant is female-sterile. "Roll-leaved fertile" shows a slight degree of female sterility; in addition, some spikelets produce supernumerary pistils, which however are always non-functional. In "partially sterile" some spikelets are female-sterile. "Awned-sterile" plants are male-sterile, the pollen grains being abortive. In "paleaceous sterile" and "roll-leaved paleaceous sterile," anthers and ovules are seldom formed, and both when they appear are non-functional. All these forms are explained as resulting from recessive gene mutations.

Among red raspberries (*Rubus idaeus*), Crane and Lawrence (1931, 1938) found plants which were functionally ♀, with abortive stamens; others ♂, with suppressed pistils; and still others neuter with organs of both sexes rudimentary. Their hypothesis assumes a gene *M* for maleness, and another, *F*, for femaleness. The recessive genes *m* and *f* determine, when homozygous, a suppression of the corresponding sex organs. Hermaphrodites may be *MMFF*, *-MFF*, *MmFf*, or *MMFf*; ♀♀, *mmFF* or *mmFf*; ♂♂, *Mmff* or *MMff*; neuters, *mmff*. Lewis (1939) finds "sepaloidy"

(petals and stamens partly or entirely replaced by sepals) to be^{res-}parently simply recessive to the typical condition.

In plants of a "half-sterile" type which appeared in Sirks' (1931) cultures of *Vicia Faba*, pistils were absent and the greatly reduced stamens produced but small amounts of pollen. The mutant character proved recessive.

THE MECHANISM OF "SEX-DETERMINATION"

As appears from the citations on preceding pages, discussion of the genetic basis of sex-expression in angiosperms has been based largely upon one or another of the theories propounded by Bridges (recently summarized, 1939), Goldschmidt (in latest form, 1934), and Correns (1907 and later).

Bridges conceived of numerous genes tending respectively toward femaleness and maleness, located on many or all of the chromosomes of a species. Those borne by the autosomes have a net ♂ tendency, those on the X chromosome a net ♀ tendency; the Y (in *Drosophila*) bears no genes affecting sex. Sex is determined under ordinary environmental conditions by the ratio of X's to autosome sets.

Goldschmidt's theory is based upon his studies of *Lymantria* in which, as in other moths, the female is heterogametic. Restating his conception in terms of the condition, more common in metazoa and angiosperms, of heterogamety in the male, it implies that femaleness and maleness are represented each by a single factor; the female factor is a gene, or a group of closely linked genes, borne on the X chromosome; the male factor is cytoplasmic. But factors of both types may occur in varying strengths (valencies). Hence sex is influenced, not alone by the proportional numbers of the respective factors as in Bridges' theory, but also by the balance determined by the valencies of those factors.

Correns' theory, already discussed in some detail, involves three types of factors influencing sex-expression. This idea was formulated at a time when sex chromosomes were unknown in plants and little known in animals. While different notions have been advanced on this point, it has been often suggested that at least one of the sex-tendency genes (Correns' α and γ) is borne on the X chromosome, and that the sex-potency (A , G) and sex-influencing (Z) genes may well be on the autosomes.

with 1. is evident that these three theories are not fundamentally contradictory. Genes affecting sex-expression may be numerous, some or all of them may conceivably exist in different valencies (or, what may amount to much the same thing, as series of multiple alleles), and different genes concerned may differ in the nature of their phenotypic effects. The last-mentioned point is Correns' important contribution to the discussion; although his classification of genic effects was necessarily provisional. Various attempted genetic formulations have in fact involved combinations of two or of all three of these theories.

A difficulty that has prevailed in the consideration of this problem has been the persistence of a traditional conception of sexual differences as in a category distinct from other individual differences. Bridges, Goldschmidt, and Correns all emphasized that sexual characters must be explained on a genic basis similar to that underlying other characters. But their terminology betrays the persisting influence of the traditional concept. The difficulty and resultant confusion of thought become evident if largeness and smallness are thought of as constituting a bipolar system in the same way as femaleness and maleness are considered to compose such a system. Every gene influencing size would then be classed as one for largeness or one for smallness—instead of as a factor affecting (in an angiosperm) number or length of internodes, primary thickness of stem, occurrence, extent, or non-occurrence of secondary thickening, and the like. Evidently, under the influence of such a conception, the analysis of size-inheritance would be even more difficult than it now is.

The possibility of a more workable analysis of sex-inheritance appeared when R. A. Emerson (1924) announced that some 9 mutant genes had been recognized which affect sex-expression in maize; that each of these produces its own particular effect; and that some of them were found to be located on different chromosomes. Now, as has been seen, many more such genes are known, distributed over at least 9 chromosomes, and each producing its own particular effect upon sexual structures or functions. As Emerson pointed out, it follows that the normal (usually occurring) allelomorphs of all these genes are essential to the typical sex-expression of the species. Since, under present limitations of genetic method, a gene can be recognized only when it mutates,

many still unsuspected may well be concerned in the sex-expression of *Zea Mays*.

In no other angiosperm has genetic analysis been carried to an extent approaching that reached in maize. However, considering together all those species studies on which have been cited in the present paper, mutations affecting sexual characters have occurred which substantially parallel most or all of those observed in maize. It appears, therefore, that the genetic mechanism underlying sex-expression must be of the same general complex nature in all angiosperms. This implies that the sexual condition established for any presently existent species is conditioned by the interaction of many different genes distributed throughout the chromosome complement. The genes play each its part in determining, under a given environment, the degree of development or non-development, of functioning or non-functioning, of pistils and stamens and of their parts, as well as of other organs.

In addition to the numerous genes thus involved, and their arrangement upon the chromosomes, it appears that an ultimate analysis of factors influencing sex-expression must take into consideration the specific composition or structure of the cytoplasm. The importance of cytoplasmic constitution is suggested by the results of several of the studies mentioned on previous pages.

In the development of varied sexual conditions from that of hermaphroditism, numerous mutations have appeared and become established, usually in homozygous form. In certain cases, heterozygosis of a particular gene pair has persisted, as probably in the establishment of gyno- or androdioecism. In dioecious angiosperms, one pair has remained heterozygous in one sex (usually the ♂); other alleles being in general homozygous, this pair has become completely or nearly completely determinative for the sex of the plant, thus lending a misleading appearance of simplicity to sex determination.

Light was shed upon the evolutionary possibilities suggested in the preceding paragraph when D. F. Jones (1931, 1934) and R. A. Emerson (1932), by intercrossing mutant forms of maize, produced permanently dioecious races. Jones obtained plants homozygous for the recessive genes "silkless" borne on chromosome 2, and "tassel seed-2" on chromosome 1. These plants are ♀. His ♂ plants are likewise homozygous for silkless but heterozygous for

tassel seed-2. Matings of ♀♀ × ♂♂ produce offspring of the same two classes as the parents. Dioecism in this case is as nearly perfect as in most species classed as dioecious; occasional ♂ structures appear on some ♀ plants, and *vice versa*. There is evidence (Jones, 1939) of differences between families in the degree of sex-separation.

Emerson's dioecious strains are two. In one, the ♀♀ are homozygous for the recessive genes barren stalk-1 (chromosome 3) and tassel seed-2 (chromosome 1); the ♂♂ are homozygous for barren stalk-1 and heterozygous for tassel seed-2. In the other case, ♀♀ are homozygous for barren stalk-1 (chromosome 3) and heterozygous for the dominant mutant gene tassel seed-3 (on an unknown chromosome); ♂♂ are homozygous for barren stalk-1 and for the normal recessive allele of tassel seed-3.

Thus from a monoecious species, by appropriate selection of mutations, have come two different dioecious strains in which the chromosomes of the first (longest) pair function as sex chromosomes; and a third strain in which probably a different chromosome pair functions similarly. In two cases the females are homogametic, the males heterogametic, as in most dioecious species; in the third, it is the females that are heterogametic, as in *Fragaria*.

These experiments demonstrate how dioecism may arise—not, of course, precisely how it has arisen in nature. They show that, while dioecious species are alike in the presence of a single determinative gene pair, it is not necessarily corresponding genes that control in different cases, nor do the chromosome pairs carrying the determinative genes necessarily correspond in different species. They show how particular chromosomes may have come to function as sex chromosomes; but the question, often discussed, remains open as to how a visible differentiation has in so many instances come about between the members of this particular pair.

It is significant that a gene or gene pair selected as determinative in each of these experiments—tassel seed-2 when homozygous or tassel seed-3 when heterozygous—is epistatic to the other selected pair—silkleless or barren stalk-1. The heterozygosis of the determinative pair in one sex, and its homozygosis in the other, produce exactly the results postulated by Correns for his sex-tendency genes or realizators. However, the discussions based upon Correns' formulation have tacitly assumed that the "realizators" belong in a

category distinct from other genes affecting sex-expression. The results with maize show this assumption unfounded; these genes are characterized by epistasy it is true, but epistasy is by no means a rare or distinctive peculiarity.

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REGENERATION AND VEGETATIVE PROPAGATION

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Soil Conservation Service

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I. INTRODUCTION

During the past few years the botanical world has been startled by a remarkable expansion of research into the realm of growth substances. This is particularly true for the theoretical and practical aspects of vegetative propagation—the field of this article. Hence any review of regeneration today certainly should include a

considerable discussion of phytohormones, whatever the terminology employed.

Nevertheless, this article is definitely not a review of plant growth substances. Several recent books and articles (24, 31, 49, 117, 149, 178, 218, 258) have covered this field far better than the capabilities of the present reviewer permit. The subject of growth substances is concerned primarily with so-called "normal" growth, including, especially, tropic and nastic movements, which scarcely belong in the strict realm of regeneration. The reader must look to some of the above cited reviews for adequate presentation of the chemical nature of these various substances which in minute concentrations are capable of markedly affecting plant growth.

Even with these delimitations, the task of reviewing recent theoretical and practical work on plant propagation has necessitated the assembly and study of a vast literature. For while the number of papers dealing with various aspects of growth-promoting chemicals is enormous, it seems that there has been no decrease whatever in research on the morphology, anatomy and physiology of various regenerative phenomena apart from the hormone angle.

Thus the first part of this paper will review various anatomical, physiological and practical reports on regenerative and propagation problems apart from growth substances; the second will consider the various theoretical and practical problems tied up with those materials; and finally, some consideration will be given to more or less miscellaneous studies (only some of which involve application of growth-promoting materials) on the lower plants and on leaf cuttings.

II. INVESTIGATIONS ON REGENERATION NOT INVOLVING APPLICATION OF GROWTH SUBSTANCES

A. Anatomical and Physiological Studies

Apart from studies on growth substances, many excellent and somewhat diverse reports have appeared the past few years on the anatomical and physiological changes involved in wound and propagation responses.

Interesting observations on periderm formation were reported by Wright, Peacock and Whiteman (270) in studying the best method of handling seed pieces of potato tubers. The most satisfactory results were obtained with high humidities and temperatures

of approximately 60° F., under which conditions growth of molds was restricted and the cut surfaces showed relatively small tendency to crack. Werner (259) also studied wound healing in potatoes, paying particular attention to the reactions following exposure of cut tubers to sunshine. He found that in general, formation of suberin and periderm proceeds faster in tangential than in radial cuts. In no case did a wound periderm appear to be formed immediately beneath the old periderm, although the cells in this region were the first to suberize.

Merry (166) found that the endosperm of wounded *Crimum* seeds responded with formation of a periderm, much the same as in potato tubers.

Woodhead (268) followed chemically the process of wound healing in *Kleinia*, finding that in the neighborhood of the wound potassium and calcium disappeared but slowly, phosphorus and magnesium rapidly. In general, phellogen activity began in those cells which had become depleted of calcium malate, phosphorus and magnesium. There seemed to be no accumulation of substances prior to the appearance of phellogen activity.

Bloch (18, 19, 20, 21) studied wound healing and air-root initiation in various monocotyledons, observing that unusual amounts of calcium oxylate deposits appeared shortly after wounding.

Heubel (102) noted that in tea the formation of wound gum following primary wounds was greatly dependent upon the amount of starch present, and that periderm on the callus appeared only with humidities of 90–96%, true cork only with humidities of 50–55%.

Garms (72) and Ulrich (247) carried out extensive studies on the processes which followed wounding of fruits on a number of common horticultural plants. Ulrich emphasized that only a fruit in full growth is capable of callusing; if too young the injured fruit drops, if too old it rots. He also emphasized that the presence of water vapor exerted very marked inhibitory action upon lignification, suberization and cell division.

Hemenway (101) found that in *Carnegiea* external and internal injuries were followed by development of a cork-like tissue composed of thick-walled heavily lignified cells alternating with thinner layers of thin-walled suberized cells.

Stoutemyer (223) emphasized the two distinct growth phases in apple trees, and that stem cuttings of wood in the mature phases

were very difficult to root without special treatments while those in the juvenile state rooted readily. This condition was manifested especially by thinness of the leaves and a small amount of pubescence. Juvenile shoots might be produced from roots of older trees, apparently always from adventitious buds. The change from juvenile to mature form does not seem to be related to secondary thickening but rather to certain biochemical factors not understood.

LaRue (135) found that *Eleocharis rostellato*, which fails to set viable seed, reproduces through the growth of buds at the tips of the culms.

Carlson (40) and A. J. Smith (212) studied anatomically the origin of roots on stem cuttings of coleus; Carlson (41, 43), their origin in rose and willow; Wolfe (267), in *Cotoneaster*; and Connard and Zimmerman (53), in *Portulaca*.

A. I. Smith (211) found that the adventitious roots in stem cuttings of *Begonia*, which originate either in the interfascicular or fascicular cambium, are always closely associated with the rays. He considered that the root primordium dissolves its way through the cortical cells of the mother tissue, thus forming a surrounding pocket even before the histogens are well differentiated on the new primordium. This question, however, has been considered by other workers without any thorough agreement. As Priestley and Swingle pointed out in 1929 (188), it seems very doubtful that roots ever emerge by digestion, it being much more likely that emergence results from actual rupture of the mother tissue.

Naylor and Sperry (176) found that in *Chlorophytum* (Liliaceae) new plants were produced at the nodes and apex of the stolons as well as in the axils of the leaves. The roots of the young plants were initiated from thin-walled parenchyma near the vessels, the balance of the new plant arising from the meristematic cells which seemed to be wholly undifferentiated at the start.

Hammond (93) studied root and bud regeneration on the water plant *Podostemon*, finding, in addition to the complete replacement of the original root growing point, many other interesting and characteristic aspects of regeneration.

Rodger (197) studied wound healing in various submerged plants, finding that periderm formation occurs, although very much slower than in land plants. Quite obviously here we have a simple

question of water and oxygen relationships, as was discussed at length for apple and willow by the reviewer in 1929 (230). With monocotyledons, Rodger observed no periderm formation following wounding.

Dehay (59) found that roots within the hollow stem of old poplar trees, particularly pollards, were characterized by especially large fibers and vessels.

Carlson (42) found that the comparatively rare phenomenon of exogenous formation of adventive shoots from root tips, accompanied by formation of exogenous adventive roots from these shoots, occurs as the normal habit of growth in the orchid *Pogonia*.

A number of workers have continued their studies upon the internal electrical polarities as manifested during regeneration; for example, with decapitated *Phaseolus* plants, Rehm (191) found marked increase in potentials in the node directly below the point of decapitation, with no effect upon the third node.

Mendel (165) reported the formation of adventive buds on the hypocotyl of a decapitated seedling of *Annona muricata*, considering this the first instance of its kind yet reported for a woody plant.

Vickery (252) studied natural vegetative propagation in two New Zealand species of *Drosera* which grow by means of subterranean tubers. These are formed from "droppers" which arise from axillary buds of young seedling.

Potzger (185) listed 22 species of conifers known to reproduce vegetatively.

Dorfmüller (60) investigated the effect of light upon rooting of *Tradescantia* and other Commelinaceae, both with and without auxin treatment. He found that while the different species varied, all showed marked inhibition of root initiation and early growth when light was applied directly to the stem, and concluded that the light acted as an obstacle to the tendency of the (natural or applied) auxin to increase the flexibility of the cell wall.

Kausche (122) studied coalescence in budded plants of *Hevea*, finding that in general the process proceeded much as with plants worked with by previous investigators of grafting. It is interesting to note that latex vessels arise very early and usually with no direct connection with those of either stock or scion. Once these mother cells are formed, subsequent latex vessels arise from them, similar to their formation in the seed.

Mendel (164) reported a comprehensive series of studies on the citrus bud union. In general, he found callusing in this genus similar to that in other plants as described by numerous earlier workers. A complicating factor here is a tendency to form wound gum. Apparently, regeneration does not occur either in the older xylum or the pith, even though these cells remain alive. He reported that, contrary to Sorauer's earlier reports, the first callus formed is not destroyed but becomes transformed into permanent tissue. Thus the uniting can not be separated into the two stages of "primary connection" and "definite union". As a practical conclusion Mendel recommended that citrus should be budded "without wood" whenever possible and with a large shield.

Went (255) studied coalescence in *Pisum* grafts. He found that no growth of the scion took place until actual union had occurred through regeneration of the vascular bundles. Since stem-elongation, leaf growth, stipule growth and petiole growth varied according to the varieties used as understocks, he concluded that each of these processes is affected by a different factor or set of factors which come from the understock and move through the living tissues only.

B. Tissue Cultures

An important milestone in botanical research was reached in 1934 when White (260) pointed out the potentially unlimited growth obtainable with excised tomato root tips in liquid media. He reported that from one single isolation he had grown the original centimeter of tissue to a length of 40,000 cm., thus demonstrating that the nutrient solution employed had been adequate for all growth requirements, replacing in every detail the food materials normally provided the root by the top. His work has been confirmed and extended by a number of workers, especially Robbins and Schmidt (193).

White himself has considerably extended this work along two lines. In 1938 (261) he described the results of his attempts to grow isolated roots of various other dicotyledons. As good or better results than those obtained with *Lycopersicum* were reported for *Raphanus*, *Brassica*, *Medicago*, *Trifolium*, *Vicia* and *Petunia*. Eleven additional species were found to grow at slower rates, and a number of others indicated that their culture could be effected

more or less readily. Hence, he concluded that all species of dicotyledons probably can be grown from root tissue cultures.

As an outgrowth of the work with excised roots—which is a type of experimentation actually more correctly designated as *organ* culture than *tissue* culture—White (262) recently reported that he had succeeded in maintaining indefinitely callus tissue obtained from a hybrid of *Nicotiana*. Over a period of 40 weeks he grew this material in an environment similar to that used in the cultivation of excised roots, renewing the culture each week by a new passage. The cultures showed no evidence of differentiation or polarity, and thus satisfied the two main requirements for a true tissue culture by remaining undifferentiated yet capable of unlimited growth.

Other experimenters with various types of tissue cultures include Bonner and Addicott (25) who grew *Pisum* roots, and LaRue (136) who used half-millimeter pieces of various parts of embryos of *Radicula*, *Taraxacum*, *Lactuca*, *Chrysanthemum*, and *Lycopersicum*, and grew them into complete plants.

Closely associated with the work described above are the various reports on artificial cultivation of plant embryos. Tukey, in a series of reports between 1933 and 1938 (244, 245), investigated the conditions necessary for artificial culture of various *Prunus* embryos and emphasized the value of such work to the plant breeder in carrying through material which otherwise would be lost. LaRue (137) grew to the seedling stage immature embryos of a considerable number of species. Bonner and Axtman (26) worked especially with *Pisum* embryos, but more as test plants in studying the reactivity of various growth substances than as a means of determining the general nutrient conditions required for embryo growth.

C. Wound Hormones

Since Haberlandt's experiments of many years ago (89), numerous workers have endeavored to determine the role of injured and dead cells in the chain of regenerative phenomena following injuries to plant tissues. Some of the most extensive recent experiments along this line are those of Beth (16) who carried on emasculation and pollination experiments, both with and without injury. In particular, Beth was studying Haberlandt's wound hormone theory

from the standpoint of the experimental production of adventitious embryos, but he could find no evidence confirming the necrohormone theory of adventive embryos and concluded that those Haberlandt found had arisen not as a result of injury and wound hormone action but as a result of pollination due to faulty emasculation. Other workers, including Badian (9), have found more or less confirmation of Haberlandt's hypotheses.

Bloch (20) studied wound healing and cork formation in air roots, finding meristematic activity closely combined with degeneration and necrosis, particularly under conditions which would permit the degenerative products to enter the cells and alter their chemical properties. Bloch's conclusions, however, seem to be essentially those expressed by the reviewer in 1934 (232), that the physical effects of these degenerative substances combined with new oxygen and moisture relationships probably are more important than those brought about by the actual substances released by the injured cells themselves.

Bonner and English investigated wound hormones and in several papers (27, 28, 63) reported a method for the purification of the specific chemical which they called traumatin. This work is summarized in their 1938 publication (28). The substance which they obtained in an almost if not completely pure state, occurs in a wide variety of plant and animal sources, but they emphasized that there may be many other wound hormones. Although these workers largely confirmed Haberlandt's hypothesis with regard to wound hormones, they concluded that the "leptohormone" from the phloem seems to consist principally of nutrient sugars. Chemically they found that traumatin, which is heat-stable, has a formula of $C_{11}H_{17}O_4N$ for the monomethyl ester. Although this is supposedly a true wound hormone, it is surprising that in its extraction from bean pods there is nothing to be gained by wounding the cells.

Loofbourow and Dwyer (153, 154) found that yeast cells injured by ultra-violet light yielded heat-stable materials which markedly stimulated cell proliferation. It was quite evident that this wound substance was released from the cells as a physiological response to injury rather than as cellular destruction products; it was a complex mixture similar to coenzyme in that it did not contain either protein or sulphur. They found that the application of indoleacetic acid to yeast brought about a similar release of substances which

they interpreted to mean simply that this was toxic throughout a wide range of concentrations.

Orsos (179) found that the so-called wound reaction, including both division and enlargement, could be brought about under sterile conditions through various protein decomposition products, including heat-coagulated kohlrabi; the first response observed was an increase in the pH within the cells. He also found that the so-called "leptohormone" was a protein decomposition product, and concluded that tyrosin is the characteristic effective substance in each case since this was found experimentally to be capable of bringing about the wound reaction.

Continuing the senior author's long series of studies, Hammett and Chapman (92) reported results and conclusions which bear heavily upon the entire growth substance field, but especially upon wound hormones. Those authors insist that to come in this category, the compound must not only stimulate cell division but also must actually be produced by a wound. They feel that "the only compound so far uncovered which fulfills these requirements is the sulfhydryl group."

These diverse results and conclusions emphasize that notwithstanding the excellent work reported, Haberlandt's hypotheses of many years ago are still neither confirmed nor refuted.

D. Practical Propagation Experiments

Although a large part of propagation research during the past few years has been concerned with growth substances, many studies have been reported dealing with other phases.

One of the problems which has long been under attack is the question of suitable understocks for growing commercial tree crops, in particular the apple and other fruits, and cacao, coffee and rubber. The outstanding work of the East Malling research station is being continued, with numerous comprehensive reports appearing from time to time on the relative desirabilities of the various root stocks for the standard English varieties of fruits. As an example of this work can be mentioned the report of Beakbane and Renwick (12) on the anatomical differences observed in wood of Type IX stock, both unworked and when grafted with 26 different apple varieties.

In the United States, Yerkes and Sudds (279) reported preliminary results on an extensive apple stock experiment, showing that very striking differences were already in evidence after only five

years in the orchard, both as regards the influence of individual understocks themselves and the adaptability of top varieties to a wide range of understocks. It is particularly interesting to note that there was no appreciable increased uniformity among clonal root stocks over seedlings.

Work with the *Hevea* rubber plant in the East Indies continues (122), but apparently the basic question is still unsettled as to the comparative influences of the root and of the top in determining latex yield. Likewise, it is still to be shown to what degree high yielding properties of individual trees can be propagated by vegetative means.

Gibbins (75) found in the propagation of coffee that softwood cuttings were more suitable than hardwood, and that vegetative propagation of this plant was not feasible at all without the use of special propagating cases. He reported that mound layering and marcottage were not as practicable for clonal propagation as pegging down terminal stems of younger plants, and considered that budding was superior to grafting, largely as a matter of practical convenience. Mayne (159) also emphasized that the problems of the vegetative propagation of coffee were still far from solved.

Pyke (189) and Cheesman (46), working with cacao, reported only partial success with any type of vegetative propagation. Cheesman emphasized that the different forms of vegetative shoots must be carefully considered in the selection of wood for budding or for use in layering or cuttings. In particular, he discussed the broad aspects of the problem, emphasizing that the yield of an individual tree is no true criterion of the sort of budded offspring it will produce. It seems evident that the situation with cacao (as with rubber and coffee), with regard to vegetative propagation, is in a very unsatisfactory state, awaiting some method of propagation capable of cheaply and rapidly increasing the superior yielding strains. Although these plants have been studied slightly in an exploratory way with growth substances, apparently the propagation of all three is still to be worked out.

Goehde (78) studied the importance of correct sand for propagation, a type of practical study which has been under way since time immemorial. He concluded that the acidity of the sand was the most important consideration, finding that, in general, a pH of 6.0 or less was to be preferred, and emphasizing that many chemicals

used in the soil as disinfectants, *etc.*, have a tendency to increase the alkalinity. Woycicki and Terpinski (269) showed that the relative moisture of the sand had a marked influence upon rooting. They found that sand with only 25 per cent water-holding capacity was unsuitable since most of the plants withered in spite of frequent syringing. They considered 50 to 75 per cent water-holding capacity the best, but this differed somewhat among the various types of plants used. These workers also showed that internodal cuttings of dahlia and pelargonium rooted quicker and better than nodal cuttings.

Iljin (109) and Vekhov and Iljin (250) studied the influence of various factors on the rooting of cuttings, particularly softwood cuttings of trees and shrubs. As have numerous other workers, they found that the age of the plant from which cuttings were taken had a marked effect upon the percentage of strike, the younger plants always being preferable as a source of propagative wood.

Yerkes, Scott and Swingle (278) followed commercial manetti rose stocks from the stage of summer growth, through the hardening-off process in the fall, into the dormant winter conditions; later they grafted the plants dug at various times. They found that success or failure as understocks in commercial rose growing was dependent upon the stage of maturity of the stock at the time of digging, and concluded that many cases of unsatisfactory results reported with apparently well-grown manetti stocks were really due to digging in an immature condition. They also showed that a simple iodide test on the older stems could be used as a safe criterion for digging, abundant starch outside a relatively dormant cambium signifying satisfactory maturity.

Leslie (144) described an ingenious method for obtaining fruit trees on their own roots. He found that in bench-grafting apple trees, the piece of rootstock used could be inverted without affecting the stand of the grafts; although growth was retarded at first, the scions were much quicker forced upon their own roots, following which satisfactory growth occurred.

Upshall (248) found that apple root cuttings in the greenhouse yielded much better shoot and root growth if planted with their tops exposed.

Kwasnikov (131) reported a comprehensive physiological and practical study on the propagation of chicory by root cuttings.

Lugovoy (155) studied the correlation between type and stage of lenticel development, with ability to root as stem cuttings, and found that tree and shrub species characterized by a particular open type of lenticel structure were readily propagated by stem cuttings.

Using hardwood cuttings, Wierszyllowski (263) was able to root various seedlings of *Pyrus* but was unsuccessful with clonal material of the named varieties.

Mirov (168) pointed out the desirability of employing vegetative propagation in order to increase individual plants of white pine resistant to blister rust. In his own preliminary experiments he showed that without employing any chemical stimulants it was possible to root cuttings from 10-year-old trees.

A number of workers have studied callus and wound healing phenomena from the standpoint of practical horticultural operations, especially healing of pruning wounds.

Paterson (180) investigated callusing of pruning wounds in Norway spruce from the standpoint of mine-prop breakage, and concluded that much less damage was done to the resulting lumber if the branches were live-pruned.

Marshall (157) followed through two growing seasons the callus developments in *Acer* and *Quercus* trees experimentally wounded throughout the year, finding that wounds made in the early spring developed callus of more desirable shape and greater area and consequently healed more rapidly. He also observed that a single coating of shellac applied immediately after wounding markedly aided callus formation, and noted that the vigor of the individual trees was reflected in the vigor of callus formation.

Collins (52) also considered the practical aspects of pruning horticultural plants and emphasized that spring is the best time for major pruning and tree surgery work.

Rebello (190) followed Marshall's methods, but with fruit trees, and obtained similar results. Using various wax mixtures instead of shellac, he found that these interfered with suberization and callusing, and that wounds so treated showed a greater tendency to crack.

Cairns (37), studying the basis for the extreme persistence of New Zealand ragwort (*Senecio jacobaea*), found that the adventive shoots which form following decapitation arise endogenously in the pericyclic phellogen in the usual manner; however, there is an exceptionally abundant supply of nutrient solution furnished the

meristematic cells of the pericyclic phellogen, the injured root heals especially rapidly, and hence bud initials are rapidly formed. Sodium chlorate, when used as a weed killer, is not translocated any distance from the crown, and the absence of an endodermis and the normal cortical layers facilitate diffusion into the surrounding soil of the herbicide, so that it is largely ineffective against this pest.

Jimenez (113) studied anatomical and physiological processes during callusing and rooting of *Ceiba*, *Bixa* and *Sandoricum*. In the first (kapok), callus formation was very rapid, especially with cuttings taken in full growth; bark, pith and cambial regions were all active. Roots formed on the wound callus at the basal ends, originating in the newly-formed phellogen below the suberized layer. With the other two plants better results were obtained by ringing portions of the bark some time previous to planting. Jones and Beaumont (116) likewise found, with *Macademia* and litchi, that ringing stems a few weeks before they were to be used as scionwood, greatly increased carbohydrate accumulation and take of grafts.

A very good resumé of vegetative propagation of tropical and subtropical plants, including especially understocks, is given by Feilden and Garner (65).

III. INVESTIGATIONS WITH GROWTH SUBSTANCES

A. Chemistry

Broadly speaking, the same substances found to influence growth, in general, are they that have been reported as affecting regeneration.

When the term "hormone" was first applied to plants, it connoted a definite chemical substance, made in one part of the plant and transported to another where it brought about a definite result. That is, a "root-forming substance" was hypothesized as a substance which, given suitable preliminary necessary conditions, brought about only the formation of roots.

As long ago as 1934 (232), however, the reviewer emphasized that "the very definiteness and extreme multiplicity of 'specific' substances so reported has caused most physiologists to pause and ask 'what really is specific for any given process of normal or abnormal physiology, and what merely has been found to exert some effect upon the plant.'" Since then, the number of "specifics" has de-

creased, while the number of substances found to affect regenerative processes in one way or another has been greatly augmented.

The list of substances definitely reported to stimulate rooting or callusing, although largely made up of the indole derivatives, does include a number of materials which are unrelated chemically. Went and Thimann (258) state that "The primary growth-promoting activity is connected with the presence of: 1) the double bond, or aromatic unsaturation; 2) a carboxyl group, free, or if esterified, readily hydrolysable; 3) a ring system, either 5-membered (auxin *a* and *b*), aromatic (naphthyl or phenyl), or a combination of both (indole, indene, *etc.*); 4) a minimum distance of at least one C atom between the carboxyl group and the ring; 5) a very definite steric structure, since in the one case studied the *cis*-compound is active, the *trans*-compound not." (In addition to this excellent treatment of the chemistry of growth substances, see (149) for a list of all known effective materials; also (282, 285, 286) for the materials studied at the Boyce Thompson Institute.)

Another aspect of the chemical question must be considered: to what extent is the phytohormone field a question of *physiology*, and how much is it *pharmacology*? That is, are we dealing with true hormones—substances made in the plant—or are we merely studying the effects of foreign substances? Although such considerations have little bearing upon the practical aspects of vegetative propagation, they are of considerable interest in viewing the broad picture.

The evidence points towards such work being predominantly *pharmacology*—that is, most of the work with root-forming substances has been done with materials wholly foreign to the higher plants. Of the commonly used chemicals, only indoleacetic acid is known to occur in plants at all. This is very commonly found in the lower organisms but the reviewer was able to find only a single unconfirmed experiment which indicated the presence of this substance in the higher plants: Lefevre (143) reported traces of it in freshly harvested material of *Brassica*, *Asparagus* and other plants. Hence, in spite of certain marked similarities in response between natural-occurring materials and the synthetic indole compounds, we should recognize that treatment with the latter substances involves the introduction into the plant of substances wholly foreign to it. Hence, in such cases it is a definite misnomer to use the word "hor-

mone" in the sense of the animal physiologist—a substance produced in one part of the organism and transferred to another part where it exerts a specific physiological influence.

B. Morphological Changes Following Treatment

The literature dealing with anatomical changes wrought by treatment with indole compounds is almost as voluminous as the practical reports of the use of these substances in obtaining rooting of cuttings. Obviously it is impossible here to consider all of these.

Changes Following Absorption Through an Intact Surface. Dorn (61) studied the development and origin of stemborne roots on uninjured internodes, as well as ordinary cuttings treated with indoleacetic paste, and found no appreciable difference so far as origin and development of roots were concerned. He reported that even though crucifers normally form only exogenous roots at the nodes, endogenous roots could also be obtained in the internodes either by the use of indoleacetic acid or by the regular cutting method. Dorn summed up his extensive investigations with the conclusion that indoleacetic acid applied from without brings about no changes or new formations which can not also be obtained in simple cutting propagation, the adventive roots forming in the same cell layers and in the same manner as in ordinary cuttings. He considered that in normal cutting propagation root formation occurs through the accumulation of growth substances.

Chouard and Costan (50) applied indoleacetic paste to cotyledons and leaves of various plants. While in most cases only cell enlargement occurred, *Pisum* plants showed a definite swelling instead of elongation. They concluded that the effect of the chemical did not depend at all upon the direction of diffusion but rather upon the nature of the tissues stimulated.

Gautheret (74) treated young plantlets with indoleacetic acid and observed that marked thickening of root and hypocotyl occurred with concentrations below the toxic level. He noticed that especially with the root, thickening was always accompanied by a wrinkling of the surface which gave the organ a tumorous appearance. Investigating, he found this was due not to cell proliferation but to the relatively isodiametric growth of the cortical parenchyma. Thus it seemed to him that indoleacetic acid did not possess formative powers.

Lefevre (142) found that young plantlets of *Pisum* treated with indoleacetic acid reacted with a marked thickening of the hypocotyl and main root, the inhibition of the root growing point itself, and the production of numerous secondary roots. Treatment with naphthaleneacetic acid, although inhibiting the main root, produced neither thickening nor secondary roots. With *Phaseolus* similar but not identical differences were shown between the two acids.

Traub (242) treated various subtropical fruits with indoleacetic acid and others of the common stimulants and observed that dilute solutions (.01 to .001%) applied directly to the fruit retarded senescence.

Granick and Dunham (84) found that with the 43 genera which they studied, plants in the same family reacted similarly to treatment with indolepropionic acid, as regards enlargement of parenchyma, cell division, formation of callus, stimulation of cambium and root formation.

Harrison (98) found in *Iresine* that all living tissues of the stem reacted in some degree to treatment with indoleacetic acid, although neither cambium nor endodermis was markedly stimulated.

Mitchell and Martin (169) reported that application of 3 per cent indoleacetic acid paste brought about formation of galls together with development of roots in the galls—a histological response closely resembling that reported by other workers for other plants. Chemical analysis of the treated and untreated plants showed that application of indoleacetic acid greatly affected transport of materials from the cotyledons. These substances moved only as far as the points of treatment where they were used in the growth processes.

Bonner and his associates (2, 22, 23, 25, 29) have reported considerable work with the vitamin-B complex, including especially nicotinic acid and thiamin (B_1). This work has indicated that both of these substances are markedly effective in stimulating growth of *Pisum* roots, whether isolated or intact, and whether the roots are on embryos or well developed plants. Surprisingly enough, apparently the addition of both B_1 and nicotinic acid is required to obtain maximum growth.

Robbins and Schmidt (194) also worked with the vitamin-B complex and, besides confirming the marked stimulatory effect of vitamin B_1 , reported that B_6 (which normally occurs in small quan-

tities in brown sugar) is also essential for maximum growth of excised tomato roots.

Molisch (170) showed that various fruits, especially the apple, may exert marked effects upon various other plants, as by increasing rooting and callusing (including lenticel callusing) of cuttings, and initiation of bud growth; they may also strongly retard seed germination and subsequent growth and bring about precocious leaf fall. Molisch concluded that these effects were due chiefly to the presence in the fruits of thylene.

Prevot (187), in connection with his studies on regeneration in *Begonia* leaves, obtained some rather striking results on intact plants through the use of a fertilizer containing pigeon manure. Whereas normal *Begonia* leaves never develop shoots until removed from the parent plant, Prevot observed new buds arising on plants which a few weeks previously had been watered with this fertilizer. He claimed that this was the first instance of the formation of buds on intact plants due to application of special substances through the roots.

Pfeiffer (183) applied various organic acids in lanoline paste to the aerial roots of *Cissus*, obtaining similar results with each of the substances used. The first result observed was an increase in the amount of cytoplasm and in the size of the phloem and pericycle cells in the vicinity of the xylem. Subsequent divisions, first periclinal, and then both periclinal and anticlinal in the pericycle, led to the production of the lateral primordia as small masses of tissue. Later increase in size of both nuclei and cells occurred in the other tissues. Both nuclear division and cell division seemed to be strictly normal.

Williams and Rohrman (265) found pantothenic acid to have a stimulating effect on green plants, without definitely concluding whether it should be classed as a stimulant or as an indispensable material.

Bloch (21) applied growth substances in lanoline to the stem of *Tradescantia* and found that this monocotyledon displayed changes quite comparable to those which other workers had reported for dicotyledons. In general, those cells and tissues especially responsive to wounding are also particularly sensitive to growth-promoting substances.

Tincker (241) applied indoleacetic paste to tomato stems and found that within 65 hours many of the epidermal, cortical and pith

cells had attained twice the size of the comparable control cells, with many radial walls developing. He noted that lignification did not keep pace with cell division, so that the new tissue was much more parenchymatous.

Havas (100) found that application of colchicine in many cases gave responses similar to those obtained with indoleacetic acid, including initiation of roots on the stems of *Impatiens*, and also noted that radish seeds germinated in colchicine solutions tuberized much earlier. He considered that colchicine has certain analogies with the "wound hormones" or "traumatin" and concluded that its various effects are exercised through mobilization of hormones already present, alteration in polarity of hormone transport, and inactivation of some of the normal hormones.

Verner (251) has reported very definite anatomical results following application of growth substances to young apple trees. As is well known, narrow crotch angles in the framework of fruit trees are very undesirable since they frequently lead to serious breakdown of the trees. Verner noticed that when one-year nursery trees had been injured immediately above the dormant bud, as by girdling, an abnormally narrow angled branch always developed from that bud. He postulated that wide crotches should result from the action of substances which are formed in the growing points of the young tree and pass downward through the developing shoots below. He, therefore, applied growth substances in lanoline paste to the upper surface of still-elongating shoots and obtained a marked increase in the angle between trunk and shoot. After elongation of the basal portion of the shoot had ceased and the tissue had lignified, the crotch angle was permanently fixed so that neither girdling nor growth substance treatment would alter it.

Changes Following Application of Synthetic Growth Substances to Cut Surfaces. As considered elsewhere under the heading of galls, many results obtained by treating decapitated plants with organic growth substances can be expected to yield anatomical structures very similar to, if not identical with, those which characterize plant galls, particularly those produced by the crown gall organism. Hence, although a large number of workers have studied this question, the work of Brown and Gardner referred to elsewhere is of primary importance, and it will not be necessary at this place to consider papers which merely confirm their results.

Rogenhofer (198) found that application of growth substances to *Populus*, *Ligustrum* and *Ampelopsis* woody cuttings greatly increased the amount of callusing, which in the untreated cutting was inversely proportional to the distance between the buds and the cut, and directly proportional to the number of buds. The strongest callus followed application of .001 per cent indoleacetic paste to the cut end, showing that this material causes both cell elongation and cell division.

Beal studied the anatomical responses to growth substances of *Lilium* (13, 14); Goldberg (79) and Howard (107), of *Brassica*; Hamner and Kraus (95) and Kraus, Brown and Hamner (129), of *Phaseolus*; and Hamner (94), of *Mirabilis*.

Brown (32), Brown and Carmack (33), Söding (217, 218), Snow (214, 215) and Snow and LeFauu (216) paid particular attention to the anatomical changes in the cambium. Brown reported that in disbudded poplar shoots the greater the amount of living bark above a wound the greater the development of local cambium activity. He found that cambium activity resulting from wounding responds to gravity in the same way as normal cambium activity, and suggested that a wound substance, capable of propagation by cell division only, was involved in local wound cambium activity. He concluded that "hormone action appears to supply the most reasonable explanation of the quantitative results obtained."

Carrying this study a step further, Brown and Carmack determined that indoleacetic acid stimulated cambium activity for a short distance below the point of its application. They noticed especially that application at the distal end of the shoot cutting would also stimulate cambium activity around a bridged wound below. Snow found that the cambial stimulation was effective through an interposed piece of moist cloth, also that it could act on a plant of a widely different family. He, therefore, concluded the response was due to a hormone; later he reported that normal cambium growth was activated in decapitated stems and hypocotyls of sunflower seedlings through application of gelatine solutions of indoleacetic acid to their upper cut ends.

Söding introduced relatively concentrated indoleacetic solutions directly into the cambium of various trees and noted that at the place of application callus and wound wood were produced, while in the deeper layers this treatment stimulated completely normal xylem

formation. He explained this result on the basis that the growth substances affect the cambium only by inciting it to growth and division, the differentiation into vessels, fibers, *etc.*, being conditional upon other factors.

Fischnich (66) found that treatment of *Populus* with concentrated growth substances gave roots; with low concentrations, shoots. He considered it debatable whether or not the addition of growth substances alone to a completely undifferentiated and indeterminate cell group would lead to root formation.

Galls. Although an adequate presentation of the subject lies wholly outside the scope of this review, certain recent observations on galls require that some mention be made of them.

Brown and Gardner (35, 36) showed quite conclusively that application of indoleacetic and other organic acids is capable of inducing formation of galls, indistinguishable from the typical crown-galls produced by *Bacterium tumefaciens*. They concluded that the presence of the organism is not essential to the formation of galls, which are the result of interaction between the host cells and indoleacetic acid or similar auxin, and that the role of the bacterium in producing the auxin can be played by other agencies. Thus, the hypothesis of the reviewer in 1925 (229) to the effect that the symptoms known as crown gall might be brought about by agencies other than actual infection with *Bacterium tumefaciens*, seems to be well borne out.

Other workers (148, 150, 219) have confirmed this property of indoleacetic acid and other organic acids for producing galls similar to those brought about by actual infection with the crown gall organism. Levine (145) obtained similar tumors with Scharlach Red.

Berthelot and Amoureux (15) observed that indoleacetic acid was produced by the crown gall organism when grown in the presence of tryptophan, and suggested that the observed immunity of older plants might be due to the relatively small amounts of tryptophan which they contain. Duggar, Locke and Riker (62) reported that in addition to gall development, the crown gall organism on tomato plants brought about the responses characteristic of the chemical substances—epinasty, initiation of roots in the stem, stimulation of cambial activity, inhibition of axillary buds, and delayed formation of the abscission layer in old leaf petioles.

Hence, the recent work strikingly confirms the suggestion of

Küster in 1926 (130) regarding use of the crown gall organism in obtaining shoot and root initiation.

Link (147) observed that indoleacetic acid was one of the chemical agents, if not *the* agent, responsible for initiation of nodules by nodule-forming bacteria, and concluded that all plant tumors (including callus galls and nodules) are brought about by local hyperauxony.

The anatomical development of crown galls has been studied carefully, especially a few years ago in the apple where known infections were compared with the wound callus of grafts (128, 201, 208, 235). This work all fits into the later investigations described above, indicating that the effects of the crown gall organism are essentially those brought about by natural or applied growth substances.

Tubeuf (243) studied the anatomical relationships between hosts and loranthaceous parasites. He looked upon the wood islets, common in such cases, as by-products of the struggle between parasite and host tissues.

Harris and Pearce (97), reporting a series of more practical studies on apple crown gall, found that inoculation either with the crown gall organism or with indolebutyric acid had a stimulating effect upon the growth of the host tree, irrespective of the formation of galls.

Brown (34) has contributed an interesting new sidelight upon the gall question, and one which may have a decidedly practical application. Following the suggestion of Havas (99), she found that treatment of various plant tissues with colchicine resulted in (a) preventing formation, (b) inhibiting further growth, and (c) ultimately killing tumors already present—both crown gall tumors and those produced by application of indoleacetic acid.

Intumescences. LaRue, in a series of papers appearing since 1933 (132, 133, 134, 138), has carefully examined in a number of plant species (especially *Populus*) this particular type of outgrowth which might be classed as unspecialized gall tissue. He observed that these structures developed rapidly on leaves in damp chambers, although they occur in nature only on leaves which have been rolled or fastened together by insects. Of the several poplars studied he found only *P. grandidentata* and *P. tremuloides* capable of forming intumescences; here leaves of all ages except the very youngest and

very oldest were capable of forming these structures which developed either on the leaf blade or the petiole. He determined that these outgrowths are due to simple swelling of the cells, rather than increase in number. The physiological condition necessary for their development seemed to be chiefly the presence of stagnant, moist air. Submerged leaves apparently lacked sufficient oxygen. Extracts of intumescence-bearing leaves, as well as of *Rhizopus stinus*, injected into normal leaves, also induced intumescences. This suggested the injection of indoleacetic acid, with similar results. Hence, LaRue assumed that "plant growth hormones are the cause of intumescences on leaves confined in unventilated, damp chambers."

With other species of plants LaRue found that the intumescences were sometimes caused by a combination of cell enlargement along with cell division. *Eucalyptus* was the only plant in which LaRue found periderm development in the intumescences.

Carrying the study one step further, LaRue investigated internal intumescences from the tunnels of leaf-mining insects. He found that although in most tunnels no outgrowth of the remaining cells occurred, in some cases, masses of cells developed from exposed veins or from the mesophyll cells. These outgrowths usually consisted of greatly swollen but undivided cells, though in some cases division was also in evidence. Thus, except for their internal origin, they seemed in all particulars to be comparable to intumescences upon the surfaces of leaves.

Parthenocarpy. One of the most interesting forms of vegetative reproduction is that involving embryo development—a process which we usually think of as being inseparable from sexual reproduction. However, one type of polyembryony is characterized by the formation—within the tissues of the nucellus—of embryos genetically identical with the seed parent. These supernumerary embryos push into the embryo sac and in many cases win out over the true embryo in the competition for space and food. As pointed out in 1926 by Frost (68), in certain citrus hybrids the true embryo apparently is always crowded out by one or more of these adventive embryos so that vegetative reproduction by seed is always the case.

Although these false hybrids are made up of no paternal chromosome material, physiologically they may differ markedly from the mother parent. In particular, citrus seedlings derived from these

false embryos exhibit the juvenile features of true seedlings, especially as regards thorniness, leaf characteristics and seed content of the fruit. W. T. Swingle (233, 234) referred to this alteration taking place in the embryo as *neophyosis*, or embryo rejuvenation, and postulated these effects as being due to chemical substances found only in association with the embryo sac.

A recently reported series of papers (54, 69, 105) have confirmed the practical application of these changes wrought by the "magic bath" of the embryo sac, and proved that this phenomenon offers a practical means of reinvigorating certain citrus clones and of reducing the seed content of the fruits. These workers also showed rather conclusively that the changes involved are not genetic, since in time the juvenile characteristics disappear.

Recent work of Gustafson (86, 87, 88) has pointed out a further (and perhaps a more correctly designated) type of parthenocarpy. He showed that certain growth-promoting substances, including indoleacetic acid, chloroform extracts of pollen, and a number of other complex chemical substances, may induce the setting of fruits which in all respects (except possession of seeds) duplicate natural fruits. Hence his conclusion that in normal fruit production the ovary is stimulated to develop into fruit by definite chemical substances, rather than by specific plant products differing with each species. Gustafson's work has been confirmed by a number of workers including Gardner and Kraus (70); these workers compared anatomically the parthenocarpic and normal fruits and found that the two developed almost exactly parallel except of course in the one case for the absence of endosperm and embryo. Artificial stimulation of fruit development has been put to practical use by Gardner and Marth (71) who obtained setting of holly fruits by spraying with indoleacetic acid.

Polytoidy. Although the common method for obtaining plants with more than the normal number of chromosomes is by treatment with chemicals, such as colchicine (17, 177), Jorgensen's method, which has been used for many years, has recently been employed in obtaining tetraploid forms of various common plants. This consists of decapitation of seedlings or cuttings (with or without auxin application) and the removal of all regular buds: of the large number of adventitious shoots which develop, some are tetraploid. This method has been used especially by the Russian workers, not as a

means of vegetative reproduction but simply as a method of obtaining new types of plants or of regaining fertility for use in plant breeding (90, 182, 186, 206, 207). Tatarinov (236) found with *Pelargonium* far the best responses when the plants were inoculated with the crown gall organism *Bacterium tumefaciens*, while Greenleaf (85) reported the most effective treatment for *Nicotiana* to be an application of indoleacetic paste to the cut surface.

C. Practical Propagation Experiments with Growth Substances

Softwood Cuttings. The literature of the past 4 years contains so many references to rooting of softwood cuttings through the use of various organic materials that no attempt can be made here to include individual mention of these. Experiments with hundreds, if not thousands of species of plants, have been recorded in the literature and undoubtedly many other plants have been tested without formal reports. (Among the best reviews dealing with practical procedures see 104, 124, 224, 283.)

With the plant materials used, greatest success has been obtained under conditions and with types of cuttings which the propagator would class as definitely favorable. The use of growth substances, however, does not make it possible to root a "broom stick, a billiard ball, or even cuttings of all the plants at any time under any conditions." Nevertheless, very striking results have been obtained, and there is no doubt that judicious use of these materials has found a definite niche in practical propagation operations.

Of the chemicals commonly employed, indoleacetic acid is probably the most popular, though many workers have reported better success in rooting cuttings with indolebutyric and phenolpropionic acids and methyl indolepropionate. Likewise, the various salts and esters of indole derivatives have been employed to a certain extent, but the general feeling is that the particular type of indole compound used is of relatively minor importance.

Zimmerman, Crocker and Hitchcock (280) reported that carbon monoxide gas induced definite rooting responses in 27 species of plants, including root initiation as well as growth of preexisting root primordia; somewhat similar results for ethylene, acetylene and propylene were reported in 1933 by Zimmerman and Hitchcock (281). Due largely to the convenience of the organic substances, however, the gases have been employed very little in practical propagation.

The various types of plants respond differently to different concentrations of growth substances. Used on softwood cuttings, however, the limits of concentration for successful rooting seem to lie between approximately 5 and 100 parts per million of the solution.

Different workers have employed varying lengths of treatment as well as varying concentrations. However, few workers have reported success with treatment shorter than 3 hours or longer than 24. Of course, as would be expected, the lower the concentration the longer the treatment indicated. The disadvantages of leaving softwood cuttings in aqueous solutions longer than 24 hours are so great that undoubtedly this point comes in as a complicating factor. In all cases the diverse reactivities of various plants as well as differences on the same plant at different times precludes exact specifications.

The early recommendations for treating cuttings with growth substances (30) specified that the materials be applied to the upper ends, inasmuch as movement of the substances within the plant was almost wholly polar. However, common practice seems to dictate solutions strong enough to counteract this tendency for downward movement; especially when the tips of the cuttings are exposed to conditions of evaporation, the applied chemicals readily move into the stem and upward (103).

Amounts of growth substances sufficient to cause very severe injury on certain tissues may still call forth root initiation. Hence, the mere fact that roots are in evidence earlier or to a greater degree on treated material does not necessarily mean from a practical standpoint the treatment has succeeded. Many plants reported as responding to a given treatment have not been carried beyond the propagation bed: "The operation was a success but the patient died!"

In general, the results obtained by use of synthetic growth substances are chiefly increased speed of rooting and number of roots on cuttings which could be expected to root without treatment. Most of the reported results emphasize this point. However, in a number of cases plants extremely difficult to root with ordinary propagation facilities have been satisfactorily propagated by use of the growth substances (57, 58, 76, 77, 125, 126, 158, 160, 228, 237, 277). Mention should also be made of the work of Cooper (55)

and Jackson (112) in achieving satisfactory propagation of particularly difficult materials by re-treating the cuttings with growth substances a few weeks after the first treatment had failed to yield roots.

Although the various indole derivatives and other organic acids and their salts yield quite similar results, certain individual differences have been reported, in addition to the obvious quantitative differences commonly displayed. For example, Pearse (181) found that with softwood cuttings of fruit trees, indolebutyric usually gave a more fibrous root system than naphthaleneacetic acid.

Went, Bonner and Warner (257) pointed out that vitamin B₁ is a true hormone for root growth—that is, under normal conditions the quantities required are supplied by the other parts of the plant and without it no root growth is possible. They were thus able to show that under certain conditions where ample indoleacetic acid and sucrose were present, lack of B₁ becomes a limiting factor in the rooting of *Pisum* as well as certain *Citrus* cuttings.

Evaneri, Konis and Zirkin (64) reported a series of experiments using sugar and potassium permanganate, in combination with indoleacetic acid. In only one case, *Lonicera*, did they find any appreciable effect brought about by the addition of potassium permanganate. As for sugar, in certain cases this seemed to be of considerable value in aiding root formation, indicating that sometimes the limiting factor in root formation might be mere lack of raw materials.

Traub (242) tested a large number of chemical substances for their effects upon subtropical fruit plants, using especially *Passiflora* and *Bignonia* as test objects and observing the effect upon root formation. He also used other subtropical plants, finding some particularly responsive, some (including the avocado and mango) especially refractive.

Myers and Bowden (173) reported that cuttings of Kudzu (*Pueraria thunbergiana*) were considerably more responsive to treatments with potassium permanganate than to any of the commercial root-promoting substances tried. They reported an 86 per cent take of cuttings which had been treated with permanganate solution for 30 minutes (1 oz. to 8 gal. of water) as against less than half this percentage for the checks; the lots given commercial

rooting stimulants were intermediate. It is unfortunate that other workers have been unable to obtain similar high percentages of rooted cuttings with this plant which is used in such enormous quantities in erosion control work and which (chiefly because of the uncertain seed supply) is so hard to produce from seed.

Root Cuttings. It is indeed surprising, in view of the undoubted benefits derived from the use of growth substances in other types of vegetative propagation, that root cuttings seem to have been ignored almost completely in these tests. One of the very few references to the use of root cuttings is that of Stoughton and Plant (222) who used naphthaleneacetic acid on *Crambe*. Their results suggested that production of buds and roots on root cuttings is dependent upon the local concentration of growth substances within the tissues. They considered that relatively large amounts of growth substances determine the production of roots; due to the polar transport of the auxin, these usually occur only at the distal end, but such polarity can be altered by the use of higher concentrations, in which case roots appear at both ends of the cuttings. Bud production, on the other hand, seemed to be associated with a low concentration of growth substances; by removing approximately one millimeter of the tissue from the base and apex of the cuttings every 5 days for a period of 8 weeks, many cuttings produced buds at both ends. Thus these workers concluded that both initiation and subsequent growth of roots and buds are determined, at least in part, by local concentration of growth substances.

Linder (146) found that treatment of horse-radish roots with organic acids in relatively high concentrations inhibited shoot production but stimulated root production so that roots appeared in the regions where buds otherwise would.

Apparently no strictly practical tests have been reported on the use of growth substances on root cuttings. Perhaps this is due to the marked inhibitory effect of these substances upon root elongation.

Hardwood Cuttings. Although much less work has been reported on the use of growth substances with hardwood cuttings, results as striking as with softwood cuttings have been obtained. In general, the same materials have been used, especially indoleacetic, naphthaleneacetic and indolebutyric acids. The time and concentration employed are such as to counteract the lesser reactivity

and the absence of leaves—that is, a somewhat higher concentration or a longer time is recommended for hardwood than for softwood cuttings.

Among hardwood cuttings, grape (6), chestnut, oak, maple, pear (39), poplar, larch, honeysuckle (126), willow (181) and aspen (213) have been rooted.

Stoutemyer (225, 226) had unusual success in rooting a number of hardwood cuttings, especially when they were callused previous to the chemical treatment. The precallusing treatment also gave marked increase in the range of concentration permissible without injury. Following chemical treatment, he subjected the cuttings to approximately 70° F. for one week previous to planting. Although, in general, other workers have found that treatment of cuttings with growth substances merely steps up the time and amount of rooting, Stoutemyer's experiments indicate other factors are at play, for he obtained best results with cuttings of a somewhat larger diameter than are commonly employed, and he also reported that the age of the wood seemed to be relatively unimportant. He also extended his previous experiments to include talc dusts as the carrier of the root-inducing chemicals, reporting that the dust method was very much more satisfactory than solutions and gave greater latitude of dosage, for both hardwood and softwood cuttings.

Root Development in Transplanted Seedlings. Although not commonly considered as directly a part of vegetative propagation, it is of course axiomatic that the speed and degree of new root development following transplanting is of primary importance in determining the survival and subsequent growth of materials taken from the seedbed to the nursery row or permanent location. Hence, any procedure which will aid in the amount and efficiency of roots is of very great practical importance. A few workers have investigated the influence of various growth substances from this standpoint.

Tilford (239, 240) used indolebutyric, indoleacetic, indolepropionic and phenylacetic acids at concentrations of 10 to 40 parts per million, on seedlings of *Ulmus*, *Thuja*, *Picea*, *Pinus*, *Acer*, *Quercus*, *Malus*, and *Prunus*, soaking the roots in these solutions for one or two days just before replanting. He observed a considerable increase in root development, especially with indolebutyric

treatment, but his experiments were not continued far enough to determine whether actual increase in survival was obtained, though the assumption was that the increase in root development was sufficient to assure appreciably better survival.

In this same connection Chadwick (44, 45) soaked *Viburnum* and *Cotoneaster* roots in indoleacetic, indolebutyric and phenylacetic acid at dilutions of 20, 50 and 10 p.p.m. for 18 hours, obtaining definite root stimulation. He emphasized, however, that the entire question should be considered further, stressing the need for determining the extent growth substances could be applied directly to the soil or in wax coatings on the roots.

Romberg and Smith (199) reported an ingenious way for applying growth substances to pecan trees in order to obtain stimulation of root growth after transplanting from the nursery. Their method was to soak toothpicks in indolebutyric acid for 24 hours, then insert them in small holes bored in the lateral roots.

Plank (184) treated the roots of *Pinus caribaea* seedlings for 24 hours with 10 to 80 p.p.m. of indolebutyric acid previous to transplanting. One year later the treated plants showed definitely increased depth of root system, with a correspondingly increased survival.

Various other workers have attempted to obtain much the same results as those discussed above by applying the growth substances directly to the soil or by dusting or soaking the seeds immediately before planting. For instance, Amlong (7) soaked *Beta*, *Datura*, *Daucus*, *Raphanus* and *Triticum* seeds in indoleacetic acid solutions and also sprayed solutions on the young seedlings, obtaining increased growth over the non-treated controls. Of course, in such a case it is questionable whether actual root stimulation was obtained or whether the results were due to general growth stimulation.

Grace (80, 81, 82, 83) reported marked increase in growth following the addition of very small amounts of naphthyleneacetic acid, obtaining a 300 per cent increase in green weight of tops of lettuce following the application of the equivalent of 150 mg. per acre, while as minute an amount as 1/200 p.p.m. definitely depressed length growth of wheat roots without altering their total weight. He also found that sulphanilamide stimulated root proliferation. Grace cautioned against translating the increased early growth so

clearly demonstrated in the laboratory and greenhouse into ultimate yield of field crops. In all his work the indication seems to be more a general stimulation of growth, rather than an increased or more efficient functioning of the root system.

In the matter of seed treatment Thimann and Lane (238) reported that low concentrations of indoleacetic acid accelerated rate of root elongation in *Avena* and *Triticum*, while higher concentrations inhibited growth in length. They reported, however, that although treatment with higher concentrations was inhibiting, growth of the roots greatly increased both in number and length following removal of the auxin. Also, the general vegetative growth of the shoot was accelerated by the treatment.

Lefevre (142) soaked seeds of *Phaseolus*, *Zea*, *Pisum*, *Raphanus* and *Brassica* with indoleacetic acid, dichlorethylene and other materials, and noted a marked thickening of the hypocotyl and radical, inactivation of secondary roots, and (especially with *Raphanus*) very precocious tuberization.

Cajlachjan and Zdanova (38) immersed seeds of various crop plants in indoleacetic for 24 hours, finding in most cases no effect as to time of flowering and fruiting but an increase in dry weight. Loehwing and Bauguess (151) reported increases in total seedling growth of *Matthiola* plants which had been watered with dilute indoleacetic acid solutions.

In this connection mention should also be made of the work of Davies, Atkins and Hudson (56) who reported that vitamin C (ascorbic acid) stimulated germination and growth of seedlings, but only in low concentrations. Also, Lazar (141) germinated *Impatiens* plants on agar and found that after the tap roots had been removed on the 12th day a regeneration of roots took place more quickly and more extensively on those seedlings which had been treated with .0025 to .008 per cent carotene.

The striking effect of growth substances upon *initiation* of roots both in roots and stems constitutes the most immediately applicable result of the growth substance studies. On the other hand, plant roots are so extremely sensitive to both natural and synthetic growth substances that root *elongation* is markedly retarded, even by minute amounts.

As with many other chemicals, numerous investigators have reported slightly increased rate of root elongation following applica-

tion of infinitesimal amounts of growth substances. Apparently, however, as is emphasized by Jost and Reiss (118), who likewise obtained results of this sort, statistically significant increases have been observed but rarely.

Summing up the reports of applying growth substances to roots or the soil, the evidence indicates rather definitely that a general stimulation of growth, especially during the first few weeks, can be expected where optimum solutions are used. This has been observed with a number of materials, with liquid or dust application to the seeds, or to the soil, or when dusted or sprayed on the young seedlings. The actual basis for the increased growth, however, has not been documented. Although there is some indication that at least in certain instances an actual stimulation in number and length of roots has taken place, in other cases the evidence points toward a definite inhibition of root growth. Hence, it is still questionable whether the application of growth substances in this way actually helps root development in either amount or efficiency.

Budding and Grafting. Notwithstanding the striking results obtained with various chemical stimulants in the matter of rooting, and in spite of the numerous theoretical papers referred to above which have pointed out the marked effect of such substances upon callus growth, it is surprising that but a small number of workers have reported the use of such substances in budding and grafting. A few papers, however, have indicated that the indole derivatives might also have a place in this phase of vegetative propagation.

Kordes (127) used indoleacetic acid to treat grape grafts in order to hasten the coalescence processes, soaking both scions and stocks for 16 hours in .01 per cent solution immediately preceding grafting. Although much stronger root development took place on the treated stocks, he also noted prompt root development on the scions at the place of union. Another objectionable result was the too-rapid development of roots on the stock while the grafts were still in the forcing chamber. He felt, however, that these details could be controlled. Müller-Stoll (172) likewise used indoleacetic acid to obtain marked acceleration of callusing with grape grafts. Although he obtained no results when using paste, by painting, spraying or dipping the prepared scions or finished grafts in .05 per cent solution, he obtained a very desirable speeding up of coalescing. Evanari, Konis and Zirkin (64), using lanolin mixtures of indole-

acetic acid, observed considerably accelerated callus formation on grafts of *Malus*, *Vitis* and other hardwood plants.

In spite of the dearth of references in the literature to the practical employment of growth substances in grafting, apparently these are used quite commonly, especially in Germany. For instance, the instructions published by the Bayer-Leverkusen Company on the use of their Belvitan growth substance in grafting include definite directions for its use (1).

Other Types of Propagation. Zimmerman and Hitchcock (284) found that while naphthaleneacetic, indolebutyric and indoleacetic acids were all effective in stimulating root growth on *Gladiolus* corms, the type and degree of rooting obtained differed with the three acids used.

Lefevre (142) observed that *Dahlia* cuttings made late in August and treated with indoleacetic acid, unlike the checks, showed normal tuberization by October.

Vegis (249) obtained precocious sprouting of *Stratiotes luronis* by soaking in weak solutions of indoleacetic acid. He noted that whereas those treated with hot water sprouted only after several days, these soaked in indoleacetic acid showed growth within 24 hours. Except for this acceleration, indoleacetic acid seemed to have the same effect in breaking the dormant period as the hot bath method and other treatments.

Calla (39) obtained beneficial results with root-forming chemicals on marcots of various hardwood species.

* * *

Thus we can conclude that treatment of intact or isolated plant parts has been conclusively shown to call forth marked responses which, when properly used, can be of great help to the plant propagator.

D. Growth Substances and Correlations

For many years workers in experimental biology have been interested in the correlations at play between the various cells and organs of a plant. Needless to say, introduction of synthetic and natural growth substances into botanical science has not lessened interest in this type of investigation and speculation. It would be possible to review a large number of publications which reported diverse effects produced upon plants depending upon the manner and degree of application of the various organic acids. Most of these,

however, can well be summed up in the words of Chouard (48), that the growth substances "Sometimes bring about roots, sometimes shoots." Auxins certainly stimulate the first phase of activity—the hypertrophy of tissues which gives in effect an unorganized tumor—but the development of this into a root or a bud is the result of other factors.

Avery (8) emphasized that "The same substance is capable of bringing about both the same and different responses in the same and different species: that is to say, most anything may happen. It makes one lean toward the conclusion that these substances are acting as evokators—*protoplasm irritants*."

Went (256) considered that the presence of other hormone-like factors which he called "calines" were necessary in addition to auxin. He reported that without "caulocaline" (formed in the roots) no elongation of the stem takes place, while "rhizocaline" coming from the cotyledons must be present along with auxin in order to bring about root formation; similarly, "phyllocaline" is necessary for leaf growth. "The specificity in development, the decision as to whether under the influence of auxin roots will develop, or growth in length or thickness will take place, depends on the relative concentration of the various calines." Hence, Went brings us back very close to the old ideas of Sachs concerning "root-forming" and "shoot-forming" substances.

The entire field of growth substances seems to be well summarized in the following quotations taken from the very excellent review of Jost (117), which the present reviewer has translated rather freely, but he believes without essential loss of the ideas expressed in the German:

"At the beginning, it seemed as though we had found in auxin the controlling agent in cell elongation through which all secrets had been unlocked; however, today we must recognize that there are a large number of substances which work similar to auxin, and which have various additional effects on other processes.

"It must be emphasized that we have found great disappointments on every hand. Auxin is no longer the 'ruler' of the plant, but its 'servant' (287). Cell elongation, which was supposed to proceed only in proportion to the amount of auxin present, has been demonstrated to proceed in the root entirely without this material. The protoplasm, which for a time seemed to be 'shelved,' has again returned to its old importance. . . ."

IV. REGENERATION AMONG THE LOWER PLANTS

A number of papers have appeared during the past few years dealing with the lower plants and bearing closely upon the problems under discussion. For convenience, due to the specialized nature of the lower plants, these are dealt with here in one place, combining the various aspects of "normal" anatomical and physiological studies with those on growth substances.

A. Pteridophytes

Among pteridophytes, interesting results have been reported for the ferns, horsetails and clubmosses. Williams (266) studied *Selaginella*, finding that the angle-meristems could be induced to give rise to leafy shoots instead of rhizophores, if the portions of the shoot used as cuttings were deprived of the shoot apices. He considered that this alteration was due to the removal of the correlating influence normally diffusing backwards from the apex, inasmuch as cut pieces of shoot smeared with indoleacetic paste responded with the formation of rhizophores as in the intact stems. He also concluded that the presence or absence of auxin, natural or synthetic, is the effective factor in determining whether an angle-meristem shall develop as a leafless positively-geotropic rhizophore, or as a plagiotropic leafy shoot, and that the influence normally exercised by the shoot apex is in the nature of a hormone mechanism.

Barrows (10, 11) as well as Roberts and Herty (195) studied the vegetative propagation of *Lycopodium complanatum* and other club mosses. Barrows found that these could be propagated readily from the young growth, cuttings made in the spring at the time new growth was starting rooting better than fall-made cuttings. Roberts and Herty, in addition to recommending ordinary stem cuttings as a means of propagation, suggested the use of those in which roots were already present. As is brought out in their anatomical studies, cuttings of this type with "arrested roots"—characterized by small mounds on the underside of the stem—constitute another instance of pre-formed root rudiments. They found that root initiation takes place only at the meristematic stem tip. If in contact with moist soil the root emerges at once; if not, it remains in the stem and its xylem lignifies, resulting in the "arrested root" which later can be induced to resume growth, when favorable conditions are presented.

Schaffner (202) found it easy to propagate *Equisetum* from cuttings of sterile shoots.

Among the ferns, numerous observations have been reported. Akdik (3) carried out a comprehensive set of physiological and anatomical experiments on gametophytes of *Polypodium*. Albaum (4, 5) studied regeneration in *Pteris* prothallia with and without indoleacetic acid treatments, reporting that apical regions cut from prothallia regenerated their heart-shaped form and that the total area of a group of adventitious prothallia depended upon the mass of the piece from which they arose. He concluded that a growth hormone, extractable chemically, is transported through the cells of the prothallium from the apex to the base. He also found that the addition of indoleacetic acid to the isolated pieces of prothallia inhibited adventitious outgrowths. In the young sporophyte the primary leaf seemed to be the auxin-producing center. This auxin (which can be replaced by indoleacetic acid) inhibits not only the outgrowth of adventitious processes from the prothallium, but also the development of other leaves.

Lawton (140) was able to induce apospory in 11 species of ferns, finding that apogamy does not necessarily follow induced apospory.

McVeigh (162) summarized her own work and reviewed the question of vegetative reproduction among ferns in general. She found that normal vegetative reproduction from leaves had been reported in 197 species, and from roots in 34, while reproduction as a result of artificial stimuli had been reported in 32 species. Apospory had been observed in 51 species. As a result of histological studies, she concluded that plants produced normally from leaves arise from unspecialized cells at the tip or from epidermal cells; those produced from roots arise from the meristem and possibly from the cortex. Plants produced as a result of artificial stimuli originate from the epidermis, from parenchyma cells, from meristematic cells and from callus. In general, the cells involved in proliferation of ferns seem to be unspecialized or at least only slightly specialized.

Sainsbury (200) described various types of reproductive structures characterizing normal vegetative reproduction among New Zealand mosses.

Yarbrough (275) described the foliar embryos of *Camptosorus*, which are essentially like those of *Kalanchoe* (Crassulaceae).

B. Bryophytes

Among bryophytes, LaRue (132) described the regeneration of protonemata from gametophyte tissue in 24 species of mosses, 20 of which constituted new records for regeneration. He noted that species which normally produce special reproductive bodies are capable of regeneration from foliage and stem.

Here among the bryophytes might also be mentioned the fact that Montel (171) described the formation of a new regenerative thallus in the liverwort *Metzgeria*.

C. Thallophytes

Regeneration phenomena among thallophytes are so specialized that they hardly can be considered in this review. It is surprising, however, how parallel to the experiments with higher plants has run research on the algae, especially the favorite subject *Griffithsia*, (106, 203, 204). Also, Weide (254) studied the regeneration taking place in isolated cells and wounded filament pieces of *Callithamnion*, finding marked differences in polarity between materials obtained from different sources.

V. LEAF CUTTINGS

Studies on various aspects of production of new plants from leaves are of especial interest, since this phenomenon involves a peculiar combination of so-called "normal" and regenerative activities. That is, a detached leaf may be considered as "intact" in that only an infinitesimal part of its area presents an exposed wound surface; at the same time, however, since it is no longer in communication with the stem and root, it offers essentially all the problems of other types of cutting propagation. Hence, it has seemed best to consider this special type of regeneration here, including both growth-substance studies and others.

Anatomical, physiological and practical experiments with leaf cuttings up to 1931 are summarized in the comprehensive work of Hagemann (91) who carried out many experiments himself and included his results with the various records from the literature. He concluded that the capacity for shoot and root regeneration from leaves is very wide-spread throughout the plant kingdom. Summarizing the results of all workers, he showed that the ability to form shoots had been found on uninjured leaves in only 46 spe-

cies belonging to 11 families. Of a total of 1204 species tested with detached leaves, 501 had been found to yield roots alone, 25 species shoots alone, and 289 both roots and shoots. Stipules, cotyledons, scales, carpels, and fruits are capable of regeneration when used as leaf cuttings, although neither stamens nor petals have been found suitable. In the matter of conditions essential for regeneration, Hagemann concluded that only with certain species does age of leaf seem to be important. Usually the period of most rapid vegetative growth of the mother plant is the most favorable time for taking leaf cuttings, but this is by no means universal. He was quite emphatic that the wound stimulus itself is not the primary cause for release of the regenerative activity, nor is accumulation of nutrients the decisive factor; the breaking of communication between the growing point and the leaf (either actually or by physiological isolation) seemed to be of primary importance in bringing about initiation of new root or stem growing points. As indicated by the figures cited above, the tendency to form roots is very much more pronounced than the tendency to form shoots. Normal leaf-borne shoots arise exogenously from the mesophyll, while regenerative shoots may arise either exogenously from cells of the epidermis, or endogenously from sub-epidermal or deeper lying parenchyma.

Schwarz (205) also reviewed the question of leaf-cutting propagation. In addition to his comprehensive anatomical studies on cotyledons and leaves of coleus (which form roots but not shoots), Schwarz included a supplement to Hagemann's monograph and listed some 100 additional species which had been used as leaf-cuttings. Of these only half yielded roots and but seven gave both shoots and roots.

LaRue (139) extensively studied the cell outgrowths which arise on wounded surfaces of leaves under moist conditions. He found that no ferns, practically no monocotyledons, and only a few dicotyledons displayed the capacity for callus production from wounded leaves. Of the latter, *Mitchella* and *Coreopsis* produced cell outgrowths following application of indoleacetic acid. A number of species which do not develop callus on wounded leaves are capable of producing adventitious roots on those organs.

A considerable amount of regenerative work has been done on the kalanchoes or bryophyllums, especially on the old standby known as *Bryophyllum calycinum* (more correctly, *Kalanchoe pin-*

natum) and two very interesting new members of this genus which the reviewer brought from Madagascar in 1928, *K. daigremontiana* and *K. tubiflora* (231). Clamp (51) studied anatomically the normal development of the pseudobulbils occurring as a regular feature in intact leaves of *K. tubiflora*, finding that the primordia and early stages of the young plantlets are very similar to those of the axillary buds. Johnson (114) studied the structure of the similar, regularly occurring foliar embryos which arise in the serrations of the leaves of *K. daigremontiana*. Freeland (67), Naylor (174), Yarbrough (272, 273), and Stoudt (221) also studied the development of young plantlets in *K. pinnatum* and other *kalanchoes* which differ chiefly from *K. daigremontiana* and *K. tubiflora* in that (unlike the latter two) new plantlets generally rise only following isolation of the leaf from the mother plant. Jurisic (120) also studied this interesting genus and found that several species were capable of regenerating roots and shoots from isolated cotyledons, primary leaves, bracts, and leaflets. It is particularly interesting to note Jurisic's observation that *K. tubiflora* was capable of generating shoots from broken leaves but not from cut ones.

There are other studies on the anatomy and physiology of the Crassulaceae (163, 220, 221, 276). Yarbrough found that in *Sedum* the development of new plants from detached leaves was sharply contrasted with the condition in *Kalanchoe* in that roots and stems developed from adventitious primordia arising in callus tissue, rather than from pre-formed primordia. McVeigh found that in *Crassula* both roots and buds originate from epidermal cells and not from the wounded surface, concluding that this was the first reported instance where an entire individual arises from mature epidermal cells. Stoudt found that in *Byrnesia* the new plants originate from a dormant meristem at the base of the leaf, and that no development of meristem takes place while the leaf is still attached to the plant. Stoudt reported that it was possible to arrange the various species of the Crassulaceae in a definite sequence with respect to the degree of meristematic differentiation taken place by the time the parent is mature.

Yarbrough (274) reported that *Tolmiea* leaves behaved much as *Kalanchoe pinnatum* and observed that the root primordia are not formed until the second or third foliage leaf has appeared; also that detachment of the parent leaf is necessary to bring about complete development of new plantlets.

One of the first objects to be used in studying regeneration and plant propagation from leaves was *Begonia*. During the past few years a number of papers have appeared dealing with physiological and anatomical studies on these leaves. Villerts (253) reported that the capacity of *Begonia* leaves for regeneration is a heritable factor and usually dominant. This is true for both root and shoot regeneration, although each of these seems to be a separate factor with some types showing few or no roots and some few or no shoots. He also found that the isolated inflorescence was incapable of regeneration but that if a leaf were grafted on the flower stalk (thus furnishing the necessary auxin) regeneration was possible. Prevot (1934-1939, summarized in reference 187) found that formation of buds upon leaves was inhibited so long as the leaf was attached to the stem; also through various alterations of the available oxygen supply that oxygen deficiency induced the formation of buds in tissues which otherwise do not form them, in this respect confirming the early work of Harig (96), and Kakesita (121) who reported that *Bryophyllum*, *Begonia* and other plants, when subjected to conditions favoring intramolecular respiration, showed increased root and shoot formation on both intact and detached leaves.

Chouard (47, 48) treated portions of *Begonia* (also *Alloplectus*) leaves with indoleacetic and indolebutyric acid. Relatively light concentrations inserted at the apex gave increased rooting at the base followed by normal bud growth. With more concentrated solutions a polar movement did not take place and roots appeared at the apex, while new buds formed both at the apex and elsewhere. With still stronger dosages rooting was still more pronounced at the apex but bud formation was completely inhibited.

Jump (119) studied the reactions of *Ficus australis* leaves to wounding and found that in only a few cases did a definite cicatrice arise, although the cells of the spongy tissue usually proliferated markedly.

Isbell (110, 111) studied regeneration phenomena in leaf and leaflet cuttings of *Ipomoea*, *Lycopersicum* and *Solanum*. In the sweet potato she found that all types of cuttings used developed roots very quickly and although the tendency to form shoots was much less pronounced, these structures also were freely produced, particularly in the heavily pigmented varieties. Leaf cuttings of

the tomato regenerated roots from the petioles while buds formed both here and in the axils of the leaflets. Leaflet cuttings regenerated only roots. Potato leaf cuttings which contained axillary buds, converted these into either shoots or tubers.

Naylor and Johnson (175) found *Saintpaulia* capable of forming new plants from leaf cuttings of either the entire leaf, the blade, or only a portion, the roots arising endogenously from the thin-walled cells between the leaf traces, the shoots endogenously in the epidermal cells of either petiole or blade. There was no indication of either structure arising from dormant meristems.

Kemp (123), working with monocotyledons, emphasized that very few plants of this class had been regenerated from isolated leaves and that the few previously reported were all a result of regeneration from the leaf bases. He succeeded in regenerating *Gasteria*, *Drimiopsis* and *Sansevieria* from mutilated leaves and *Zamioculcas* from mutilated leaflets. He found that the roots arising from these leaves in *Gasteria* and *Sansevieria* were produced independently of the buds, while in *Drimiopsis* they were usually associated with them. Kemp concluded that either a meristem was present or else the injury was followed by certain cells regaining meristematic conditions. Although not definitely proven, it seemed to him that the faculty of bud production could be explained by the formation of the dicotyledonous or "initial" type of cork cambium instead of the limited "etagen" type characteristic of most monocotyledons. (In this connection, see Priestley and Swingle (188), pages 29 and 30.)

McMartin (161) followed the anatomical changes (including the accompanying secondary growth) in the petiole of *Acanthus* preceding and following the formation of pericyclic roots.

Mallik (156) observed the formation of roots but not of shoots from the callus arising at the base of the petiole of *Ficus religiosa*.

Miki (167) reported on the regenerative capacity of leaves as well as roots of several water plants.

Rhodes and Scott (192) studied the structural changes taking place in leaf cuttings, particularly from the standpoint of determining the source of the food supply to the new roots.

Roberts and Lawrence (196) and Wilden (264) obtained well rooted cuttings of *Dahlia* by taking leaves in the fall and plunging the petioles in sand. Roots quickly formed and the plants devel-

oped and blossomed the following summer. Wilden found that even better results could be obtained by leaf-bud cuttings taken through the winter or even as late as mid-April.

Wylie (271) studied callusing phenomena in leaves of broadleaf evergreens.

Practical use of leaf cuttings as a means of rapid increase of plant materials has been reported by several workers. This is well summed up by Garner and Hammond (73) who refer to much of the practical work reported since Hagemann's important monograph. They emphasize especially the work of Hunter (108) who used citrus leaf cuttings without success, but found that leaf-bud cuttings (that is, cuttings composed of a leaf with an axillary bud and a small portion of the stem tissue attached) rooted within a few days and developed into strong plants. Of course many more leaf-bud cuttings can be taken from a plant than cuttings of any other type; also, such cuttings occupy less space in the propagation frame and are more easily handled. Following up Hunter's work, that of Stoutemyer, Maney and Pickett (227), and that of Tukey and Brase (246) of the same year, Garner and Hammond worked extensively with the leaf-bud method of propagation, especially with *Rubus*, and obtained marked success by this method, particularly with the Himalaya blackberry, the loganberry and the youngberry. As would be expected, the use of heated frames was advantageous. They showed that whereas a good mother plant in the nursery might yield 40 new plants by the usual tip-layering method, up to 200 rooted plants could be obtained by the leaf-bud method.

Skinner (209, 210) reported good success in the propagation of azaleas and other broadleaf evergreens through the use of leafbud cuttings. Although it was unnecessary to use growth substances he found that use of indolebutyric acid increased the yield and decreased the time required for rooting.

Longley (152) also successfully used the leaf-bud cutting method on numerous species of plants, both with and without auxin treatment.

Briefly summing up these experiments with leaf cuttings we can conclude that although the capacity for root or shoot formation is very widespread and of great theoretical interest, in only relatively few instances has the leaf cutting method found practical application.

VI. SUMMARY

Theoretical and practical research of the past few years dealing with various aspects of vegetative propagation has been summarized and evaluated in the foregoing part of this paper, and studies on growth substances insofar as they relate directly to problems of asexual reproduction have been considered. Numerous more or less unrelated reports on wound healing, and root and shoot initiation in a wide variety of plants and tissues are also considered, and tissue culture experiments with root, callus, and embryo parts are reviewed. In summarizing the results we may consider them under the following four headings:

Investigations on Regeneration not Involving Application of Growth Substances. Recent extensive and somewhat contradictory work with wound hormones have as yet neither confirmed nor refuted Haberlandt's hypotheses of many years ago.

Practical propagation experiments have been particularly concerned with the question of understocks for fruit trees, coffee, rubber and cacao. With the *Hevea* rubber plant, the basic question is still unsettled as to the respective roles of the root and top in determining latex yield. With apple, some extensive experiments have disclosed no appreciably increased uniformity among clonal roots as compared to seedlings.

With rose understocks the degree of starch accumulation outside the cambium, as determined by a relatively simple iodide test, has been shown a safe criterion for maturity of plant at digging time.

Relative openness of lenticel structure has been reported a good criterion of "rootability" of trees and shrubs.

Pruning wounds made in the early spring have been found to heal quicker than those made at other times.

Investigations on Regeneration Involving Application of Growth Substances. The same substances which influence growth in general, affect regeneration.

So far as regenerative phenomena are concerned, growth substance research is a phase of *pharmacology* rather than *physiology*. That is, in applying indoleacetic acid and similar chemicals to the higher plants we are subjecting them to wholly foreign materials. Hence it is a definite misnomer to call such substances "hormones."

Treatment of intact or isolated plant parts has been conclusively shown to call forth marked responses which can offer great help

to the plant propagator. However, almost without exception, these are of the stimulatory nature—that is, their application is a very important factor in determining the *rate* and *degree* of regenerative phenomena, but has very little if any *qualitative* effect upon the regenerate. The growth substances (either natural or applied) markedly affect the cambium by inciting it to growth and division, but differentiation into vessels, fibers, *etc.*, is conditional upon other factors.

Work with bacterial galls has shown rather conclusively that actual presence of what is usually considered the causal organism, is not essential: the galls are the result of interaction between host cells and indoleacetic or similar auxin, and the role of the bacterium in producing the auxin can be played by other agencies.

Similarly, fruit formation has been shown to be the non-specific response of the ovary to indoleacetic or other auxins which are usually, but not exclusively, an accompaniment of sexual reproduction.

Practical experiments with the use of growth substances in plant propagation have been very successful, especially with stem cuttings (both hardwood and softwood) though not with root cuttings. Preliminary work indicates that the various chemicals may also have a place in stimulating new root growth following transplanting. Favorable results in budding and grafting have been reported but are not so conclusive or definite as with cuttings.

Regeneration among the Lower Plants. In general, physiological studies with the lower plants have run remarkably parallel to those with spermatophytes.

Leaf Cuttings. The peculiar combination of “normal” and “regenerative” phenomena inherent in leaf cuttings has made these very popular objects for study.

The decisive factor in bringing about regenerative phenomena in leaf cuttings seems to be the breaking of communication between growing point and leaf.

Practical application of the enormous amount of work done with leaf cuttings seems to be largely confined to the leaf-bud method of propagation.

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THE MYXOMYCETES

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The slime molds have been known and studied for over two centuries. Quite naturally, the earlier references to the group are almost entirely taxonomic. A review of this literature is beyond the scope of the present discussion. Micheli (138) illustrated and described several forms sufficiently accurately to permit recognition of the genus and in some cases the species. Other pre-Linnaean authors added to the published information on the group so that Linnaeus was able to include brief descriptions of seven species in his *Species Plantarum*, recognizable for the most part in the light of the work of his predecessors, although he himself contributed nothing (See 121). Order was brought into the treatment by Persoon (148) and Fries (55). While both Micheli and Fries noticed and commented on the plasmodial stage, they, like their predecessors and contemporaries, continued to regard the Myxomycetes as Gasteromycetes, to which group, indeed, the mature fructifications, especially of certain of the larger forms, show a striking, if superficial, resemblance. As early as 1797, however, Schrader (162) had dissented from this view.

Modern knowledge of the group begins with the work of de Bary. In 1854 (6) he noted that the spore of *Hemitrichia Vesparium** gives rise, upon germination, to a flagellate, rather than to a mycelial tube. This was confirmed by Bail (3) and Hoffmann (78). Within the following decade de Bary published a series of papers (7, 8, 9) culminating in the second edition of his monograph (10). Meanwhile, Cienkowski's studies on the plasmodium had appeared (28, 29). As a result of this work the general sequence of events in the life cycle of a slime mold, beginning with the germination of the spores to produce motile swarm-cells, the fusion of the swarm-cells or myxamoebae to form the plasmodium and the transformation of the plasmodium into the fructification characteristic of the particular species concerned was thoroughly established.

* In referring to species, it seems desirable to use current names where such differ from the names used by the authors of the papers cited. In the present instance, *e.g.*, de Bary referred to the species as *Trichia rubiformis*.

It is scarcely possible to overemphasize the clarity and precision of de Bary's papers, which must serve as the fundamental starting point for nearly all later studies. But, by reason of its very accuracy and completeness, de Bary's treatment raised other questions, such as the nature of the Myxomycetes, their relation to other organisms, the limits of the group and the manner of their nutrition, which have not yet been wholly answered. With advance in technique, other problems have appeared, especially those connected with the cytology of the plasmodium and fructification, nuclear fusion and reduction and the interpretation of the life cycle in terms of so-called "sexual" phenomena. Finally, the concept of the plasmodium as a large mass of naked protoplasm has tempted the physiologist to use it as a subject for experimental study, a fact which in recent years has brought into the foreground attempts to devise improved culture methods.

To de Bary in 1864, as to his predecessors, the known slime molds were all included within the limits of what are now referred to as the Myxogastres or Endosporeae. Very shortly, however, other organisms were discovered or reinvestigated which showed suggestive similarities to the Myxogastres. Famintzin and Woronin (48) found that the long familiar genus *Ceratiomyxa*, previously regarded as a hyphomycete, arises from a plasmodium wholly comparable to that of the Endosporeae and that its spores give rise on germination to an amoeboid protoplasmic mass that speedily becomes organized into a group of eight associated swarm-cells. On the basis of these facts, they concluded that this genus represents a special group of Myxomycetes. Soon afterward, Woronin (200) showed that the club-root of crucifers is caused by an organism resembling both the Myxomycetes and the chytrids, later publishing his classical account of *Plasmodiophora brassicae* (201). A decade earlier, Brefeld had restudied *Dictyostelium* and decided it was a transitional form between the slime molds and the mucors. In 1873, Cienkowski discovered a similar form in *Guttulina* and shortly afterward (30), in describing new rhizopods, including *Vampyrella*, repeated the suggestion, apparently made in his earlier paper, of a relationship between the Myxomycetes and Monadineae. Van Tieghem (189) seems to have been the first to have distinguished clearly between the aggregate plasmodium of these forms and the true plasmodium of the others, and suggested

that there were four distinct groups involved, the Myxomycetes "proprement dit," i.e., the Endosporeae; *Dictyostelium* and its relatives, for which he proposed the name Acrasieae; a third group represented by *Ceratiomyxa* and probably a fourth represented by *Plasmodiophora*. In his later detailed study of two members of the Acrasieae, Brefeld (18) recognizes van Tieghem's distinctions between the aggregate plasmodium of these forms and a true plasmodium, although he does not refer to van Tieghem's paper. His term, pseudoplasmodium, for the mass of united but not fused amoebae has been very generally adopted.

The extreme extension of the range of the group is to be found in the treatment of Zopf (203), who included not only the various forms thus far mentioned, but a host of other simple, animal-like organisms, the whole divided into the Monadineae and the Eumycetozoa. The former group included the Plasmodiophoraceae, the Vampyrellaceae and four additional families; the latter, the Acrasieae, the Endosporeae and the Exosporeae (*Ceratiomyxa*). De Bary, in his later treatment (13), divided the Mycetozoa into the Myxomycetes, including *Ceratiomyxa*, and the Acrasieae. Schröter (162) modified van Tieghem's treatment by recognizing the three orders Acrasieae, Phytomyxineae (*Plasmodiophora* and its allies) and the Myxogasteres, including the Ceratiomyxaceae in the last-named order as the first of its eleven families. Schröter's treatment has met with wide acceptance, especially in botanical text-books. De Bary considered the Myxomycetes and Acrasieae clearly related but did not commit himself as to which was the more primitive. Harper (74) stated "there can be no question that the Acrasieae represent simpler forms out of which the Myxomycetes have developed." Lister, in the various editions of the English monograph (117), included only the Exosporeae and the Endosporeae; Macbride (125) included *Plasmodiophora*, but this genus was omitted by Macbride and Martin (127). Lotsy (123) divided the Myxomycetes into the Sorophoreae, with aggregate plasmodium, and the Myxogastres, with fusion plasmodium, extending the limits of the latter group so that it embraced both the Plasmodiophoraceae and *Ceratiomyxa* as well as the Endosporeae in the strict sense of Lister. He quotes with approval the opinion expressed by Harper (74) that there is no phyletic relationship between the Endosporeae and the Phycomycetes, and adds that the contrasts between his two

major groups are such that any relationship between them must in all probability be extremely distant, tracing the Sorophoreae to amoeboid Protozoa and the Myxogastres to flagellates. He regarded the entire group as animal-like, and included them in his treatment only because the zoologists, "mit konstanter Bosheit" had consistently rejected them. Kent (100), however, had previously insisted on their protozoan affinities. Later, Doflein (39) included them in his class Rhizopoda of the Protozoa, and recent zoological treatments have followed him. Maire and Tison (129, 130) held that the Plasmodiophoraceae should be regarded as a distinct group between the Sporozoa and the Myxomycetes and that the Acrasieae are not closely related to them but rather to the rhizopods. This view was attacked by Pavillard (145, 146), who maintained, however, that the term Myxomycetes was without precise meaning but might usefully be employed to designate the three series, which he regarded as very probably not closely related.

As early as 1902, Olive (141) had pointed out that the pseudoplasmodium of the Acrasieae is a phase connected with fructification and that the "vegetative" stage ends before the pseudoplasmodium is formed, hence it is by no means homologous with the plasmodium of the true Myxomycetes. This view is tacitly accepted by Jahn (91) who would trace the higher Myxomycetes from the rhizopods, and by Pascher (144) who, however, emphasizes their flagellate affinities. Both authors agree in excluding the Acrasieae and Plasmodiophorales and both emphasize the highly specialized character of the Myxomycetes. In a later treatment, Jahn (92) includes a few of Zopf's Monadineae as his first order, Hydromyxaes, but excludes most of them. The Plasmodiophoraceae he regards as reduced chytrids. Schwartz (165), while recognizing the possibility of relationship between the Myxogastres and Plasmodiophoraceae, emphasizes their differences and stresses the relationship between the latter group and the chytrids. Skupienski (171) reported the eventual fusion of the amoebae in the young fructification of *Dictyostelium* (Acrasieae) into what he regarded as a true plasmodium, though for a very brief period. This has never been confirmed and must be regarded as highly questionable. Even if it should be verified, the homology between such a condition in a fructification and the assimilative plasmodium of the Myxogastres would be more than doubtful.

As has been evident in the preceding discussion, the difficulty in deciding upon the limits of the Myxomycetes is closely tied up with the problem of their relationship to other groups of organisms. One stumbling block has been the conviction, traditional rather than rational, that all organisms must be classed either as animals or as plants. Even in modern writings in which the arbitrariness of such a division is explicitly admitted, the influence of the traditional view may be recognized. De Bary, as a result of his earlier studies, became convinced that the Myxomycetes were animals and proposed for them the group name Mycetozoa, since widely adopted in zoological treatises and often used by botanists. In the subtitle to the first edition of his monograph (8) de Bary refers to them as included among the "lowest animals." In the revised work (10) this phrase is changed to read "lowest organisms" and he specifically points out that controversy as to whether they are plants or animals has little meaning. This point of view he maintained in his later writings. Haeckel (72) included the Myxomycetes as an independent group of his Protista. Cohn (32) definitely excluded them from the fungi, stating that their relationships are rather with the rhizopods or, possibly, the sponges, a suggestion later viewed favorably by Kent (100). Rostafinski, de Bary's student, in his doctoral thesis (158) referred to them, on the other hand, as equally related to the fungi and the true animals.

The influence of tradition alone would insure, of course, that de Bary's conclusions should not go unchallenged. The early opposition, represented by the discussions of Hoffmann (78), Wigand (198) and Roze (160) was mainly dialectical. Cornu (38), however, pointed out the resemblance between the Myxomycetes and certain of the chytrids and concluded that the former were as certainly fungi as the latter. This view was adopted, with modifications, by various later authors, including van Tieghem (189), Gobi (69), Cavers (25) and Cook (35). Gäumann (57) and Gäumann and Dodge (58) admit the possibility of relationship between the Myxomycetes and those chytrids which Cornu discussed, but would separate all such chytrids from the remainder of the fungi as Archimycetes. Fitzpatrick (50), on the other hand, considered that the relationships of these more or less naked and holocarpic chytrids are with the membranous and eucarpic forms and merged the two groups into the same order. An extreme extension of the meaning

of the term Archimycetes was that of Cavers (25) who used it to include the Myxomycetes, Plasmodiophorales and Acrasieae as well as the chytrids and all other Phycomycetes. The Plasmodiophoraceae and the Myxomycetes, in the restricted sense, he regarded as independent, but closely related groups and the Acrasieae as not so closely related, but all originating from simpler Protozoa. Cook (35) eliminated the Zygomycetes, but retained the other groups in the Archimycetes, pointing out, however, that the Mycetozoa, from which he segregates the Proteomyxa, Plasmodiophorales and Acrasieae, constitute a united, and, if *Ceratiomyxa* be excepted, a remarkably homogeneous group, with little to give a clue to their phylogeny. Pascher (144), as previously noted, pointed out that the Myxomycetes must be regarded as a highly evolved group, the relationships of which are most faithfully indicated by the swarm-cells. He points out that both chlorophyll-bearing and non-chlorophyllous flagellates may possess an amoeboid holozoic phase, and argued for the descent of the Myxomycetes, from which he excluded the Plasmodiophoraceae and Acrasieae, from flagellates, as one of numerous rhizopodal side-lines, not from forms represented by *Enteromyxa* and *Vampyrella*, as Jahn (91) had proposed. Pascher believed that the peculiarities of the Myxogastres are best explained on the supposition that they are rather highly developed plant-like derivatives of colorless flagellates. This opinion had been stated, although with less precise documentation, many years previously by Masee (135). Martin (131) regarded the Myxomycetes, in the restricted sense, as comprising a class of the fungi coordinate with the Phycomycetes, Ascomycetes and Basidiomycetes, the whole constituting a phylum independent of the plant phyla and possibly derived from the colorless flagellates. Copeland (37) would modify this by seeking for the origin of the Myxomycetes in the same group of colorless flagellates which gave rise to the remaining fungi, but through a different phyletic series.

Some of the difficulties in attempting to trace these relationships are partially resolved if it be admitted that it is not necessary to suppose that life has originated on the earth but once. The opposing point of view was clearly stated by Schäfer (161) and has been consistently supported by Francis (51, 52, 53, 54) in the reports of his exceedingly significant experiments, and still more recently by Lichtig (112).

SPORE GERMINATION

Numerous references to spore germination occur in the older literature (6, 8, 40, 78, 101, 128). Lister (120) noted that germination of the spores of a species of *Badhamia* was facilitated by alternate wetting and drying. Jahn (89) confirmed this in the case of a species of *Stemonitis* and attempted to classify germination by types. He observed that spores of *Reticularia* would not germinate at 37° C., but if they were kept at that temperature for five minutes, then wet and cooled to room temperature, germination was hastened. Constantineanu (34) germinated the spores of a number of species in redistilled water as well as in nutrient solutions and studied the effect of nutrient materials, temperature and other factors on the process as well as on subsequent development. Cook and Holt (36) germinated a number of species, but with results too irregular to permit of generalizations. F. A. Gilbert (62, 64, 65) agrees with Jahn that there are, in general, two methods of dehiscence, connected, however, by forms showing intermediate behavior. He lists nine species of the calcareous group in which the number of swarm-cells emerging from a spore varies from one or two to four. He attributes lack of uniformity in previous reports to various unconsidered factors, the most important being the age of the spores, the conditions under which they were formed and differences between species. Hoffman (78) germinated spores four years old; de Bary (10) some that were six years old. Smith (178), germinated spores of twenty-one species, representing all groups, from five to thirty-two years after their collection. This remarkable longevity of the spores calls to mind an early observation of Lévillé (111) who reported the activation of a sclerotium after twenty years in the herbarium. Smith also showed that multiple emergence of swarm-cells was not confined to the calcareous species, but occurred as well in a number of non-calcareous species. The most extensive studies on spore germination are those of Smart (176, 177) who germinated spores of seventy species and varieties in water and a great variety of culture solutions. All but four species were found capable of germination in water, but most species germinated better in weak decoctions of the ordinary substrata upon which they naturally occur. The optimum pH ranged from neutral to rather strongly acid and the optimum temperature from 22° to 30° C. An interesting extension of Jahn's work on the

effect of short exposure to relatively high temperature is Smart's discovery that certain spores which usually germinate by means of pores may, after such treatment, germinate by splitting. Wilson and Cadman (199) had previously found that single spores of *Reticularia Lycoperdon*, when isolated, could not be germinated, although masses of spores from the same fructification germinated readily. Smart sowed single spores of fifteen species and secured germination with twelve of them. When, however, single spores were sown in liquid in which numerous spores of the same species had previously germinated, after filtration through a sterile Berkefeldt filter, all species germinated. Mass sowings made in such liquid gave in most cases a higher per cent of germination than similar sowings in water or nutrient solutions and the time required for germination was usually less. Smart is inclined to support Wilson and Cadman's conclusion that some autocatalytic agent is secreted by the spores and that it is necessary that it be present in relatively high concentration for germination to occur. He also thinks it possible that an enzyme may be secreted, as postulated by Jahn, but would limit its action to softening the spore wall.

NUTRITION AND CULTURE

Many of the earlier students were able to grow plasmodia in quantity on tan bark and various other organic substrata, either by bringing in plasmodia from the field, by letting them develop naturally in moist chambers, or by sowing the spores on such material. In all such cultures the plasmodia are associated with bacteria and molds and under such circumstances determination of the nutrient material actually utilized is scarcely possible with any degree of precision. Strasburger (186) recommended dead stems of *Vicia Faba*, sterilized and suspended in a sterile decoction of the same, as a culture medium, and found it possible, by sowing the spores of *Didymium difforme*, to secure all stages of development in such cultures, with bacteria present only as contaminants. Lister (114) reported that the plasmodium of *Badhamia utricularis* was able to utilize the hymenium and often the tougher portions of the fructifications of various hymenomycetes, showing marked preference for some species and rejecting others. Potato starch was utilized if softened by cooking, but not otherwise. Later (115) he observed bacteria taken in by swarm-cells and digested in vacuoles, and ques-

tioned whether any nutrition was taken in solution. Čelakovsky (27) grew plasmodia by the method recommended by Strasburger and found some evidence for the utilization of starch but believed spores and hyphae of fungi were rejected. Miller (139) grew what he referred to as aseptic cultures on hay infusion, but did not eliminate bacteria. Klebs (105) cultured two species of *Didymium* on *Vicia*-infusions and seems to have been the first to employ agar in this connection. Harshberger (77) secured from sporophores of *Pleurotus* a yellow plasmodium which he regarded as that of *Fuligo septica*, although it is doubtful whether it was that species, and found it could utilize not only other agarics, but raw beefsteak, the boiled white but not the yolk of egg, and the gamba but not the stipe of *Phallus impudicus*. Pinoy (150, 151, 152, 153) believed bacteria were essential both for spore germination and for development, and was able to secure vigorous cultures associated with a single species of bacterium. Constantineanu (34) grew plasmodia on various substrata but found Knop's solution with sugars most favorable. Brefeld (18) implies that pure cultures of many species may readily be secured, but there is nothing in his statement to lead one to suppose that he had secured cultures free from bacteria. Skupienski (174) is the first to claim such cultures. He secured these by sowing spores of fructifications of *Didymium difforme* four years old in which the bacteria commonly associated with this species had died. Such cultures developed feeble plasmodia unable to fruit, but when grown with other organisms, either bacteria or molds, responded with vigor and proceeded to fructification. They would not fruit when provided with dead bacteria, and the fructifications secured with organisms other than the usual bacterium were in some cases aberrant. Skupienski, like Pinoy, regarded his experiments as proving the existence of a definite symbiosis between the slime mold and the bacterium. In a later paper (175) he notes that the nature and degree of dilution of the substratum has a marked effect on development. F. A. Gilbert (61, 63) reported that swarm-cells of numerous species were able to feed, not only on bacteria, but on the spores of various molds, provided only that these were not too large. Howard (80, 81) found that plasmodia would grow vigorously on rolled oat agar and only slightly less so on corn meal agar, carrot decoction agar and, contradicting Harshberger's experience, on autoclaved egg yolk. Watanabe (193, 194) grew plas-

modia of a number of species on plain agar to which he added various species of living bacteria and yeasts from pure cultures, and noted marked variation both in the ability of the different species of slime molds to utilize such pabulum and in the acceptability of the various bacteria and yeasts to the slime molds. Howard and Currie (83, 84) greatly extended the number of species of slime molds known to attack the fructifications of the higher fungi and showed that the mycelium of numerous species of fungi, especially wood-rotting forms, could be utilized. Camp (21) placed plasmodia on gauze or filter paper, arranged as a wick, and fed them directly by sprinkling with pulverized oats. Smart (177) sterilized, before germination, the spores of two of the species which he studied. When this was done, he found that while the resulting swarm-cells were able to ingest bacteria and fungous spores, they thrive best when in pure culture and all nutrient was in solution. He found that nutrient solutions favor fusion of swarm-cells and plasmodial development. Cohen, at the Richmond meeting of the A. A. A. S., reported securing myxomycete plasmodia in pure culture, but details are not yet available. Kambly (97, 98) germinated spores in distilled water and transferred the swarm-cells to plates of nutrient agar, securing typical plasmodia of a number of species, but with bacteria always present.

The evidence thus far supports the early belief that at least some species of Myxomycetes are able to utilize nutrient material in solution as well as to ingest concrete particles and digest them. It may be inferred that in nature both methods of securing food are utilized.

MORPHOLOGY AND CYTOLOGY OF THE FRUCTIFICATION

Microscopical study of the elements of the fructification begins with de Bary (7, 8, 10), Wigand (198) and Rostafinski (159). The first mitotic figures in the maturing fructification were described by Strasburger (187) in *Trichia decipiens*. Rosen's curious findings in *Fuliga septica* (157) in which he stated that the number of nuclei in a plasmodium is equal to the total number of single amoebae which the plasmodium contains and that the only mitosis, and that of a simple type, occurs immediately before spore formation, are properly criticized by Lister (116) who showed that the number of nuclei in a plasmodium increases enormously during the course of

its development, at least in part by mitosis, and by Harper (74) in his careful study of *Fuligo*. Conard (33), studying a species of *Lycogala*, secured results similar to those of Harper. Jahn (85, 86) followed the development of the fructifications of *Comatricha* and *Dictydium*. Krantzlin (107) studied both the morphology and cytology of various species of *Arcyria*, *Trichia* and *Oligonema*. She doubts Lister's report of amitotic division in the plasmodium, but reports nuclear fusion in the sporangium just before the final mitosis preceding spore formation. The latter is therefore regarded as a heterotypic division, the second meiotic division being deferred until just before or immediately after spore germination. Harper and Dodge (76), studying species of *Trichia* and *Hemitrichia*, find radial stages somewhat similar to those illustrated by Krantzlin in connection with the early stages of the capillitium but dissent sharply from her interpretation, pointing out that the lines she describes do not originate from centrosomes but from granules which are usually on the surface of the vacuolar cavity in which the thread develops. Harper (75) secured essentially similar results with *Didymium*. Bisby (14) studied capillitium formation in *Physarella* and *Stemonitis* and prefers to speak of the origins as capillary invaginations rather than vacuoles. Wilson and Cadman (199) claim that in *Reticularia* the pseudocapillitium is formed from degenerating and potentially sporogenous protoplasm, the amount formed being dependent in considerable degree upon the atmospheric moisture present when fructification takes place. This is significant as providing cytological evidence for the distinction between capillitium and pseudocapillitium based on morphology. Howard (80), studying *Physarum polycephalum*, confirmed Harper's view of the vacuolar origin of the capillitium. He found the two types of nuclei reported by previous authors, and interprets the small, densely stained nuclei as degenerating, while the larger, less heavily stained ones are in early prophase. For the grosser structures of the fructification, Baker (4) studied and illustrated a large number of representative species in all groups, while Emoto (46) illustrates photographically the development of the fructifications from the plasmodium. Jahn (93) and Camp (23) have made careful studies of plasmodia, bringing out anew the fact that these structures are by no means so simple as has commonly been supposed.

NUCLEAR CYCLE

Cienkowski (29) believed that the nuclei disappeared when the plasmodium is formed, but Schmitz, cited by Strasburger (187), and Strasburger himself showed that nuclei were present in the plasmodium. These authors, as well as de Bary, assumed that such nuclei were in part the persistent nuclei of the swarm-cells and in part the products of their division. Lister (116) found mitotic figures, but illustrated what he regarded as stages in direct division, which he thought also occurred. As late as 1916, G. Lister (122) repeats this opinion. Howard (82) showed that in *Physarum polycephalum* mitosis occurs nearly simultaneously in a large plasmodium and that the process is very rapid, requiring only 20–40 minutes for completion, suggesting why previous investigators had so often failed to find it. He saw no evidence of amitosis. There is no convincing testimony that direct nuclear division ever occurs in the plasmodium except in the case of nuclei which are on the way toward degeneration (199). It is possible that this is the explanation of the amitotic divisions in maturing fructifications reported by von Stosch (184).

The simultaneous or nearly simultaneous division of nuclei in the sporangium preceding spore formation, first noted in *Trichia* by Strasburger (187), has since been observed in a number of other genera (14, 33, 74, 80, 199). Harper (75) found it to be less apparent in *Didymium melanospermum*. Jahn (89) and Kräntzlin (107) assumed this to be a heterotypic division, with meiosis completed upon spore germination. Later, Jahn (91) states that reduction is completed in a single division, the first division in the spore being in no respect different from the succeeding divisions preceding nuclear fusion. Pinoy (154), working with *Didymium nigripes*, believed that the spores were of two sorts, + and –, hence that meiosis must precede spore formation, and that both sorts must contribute myxamoebae to the plasmodium if it is to fruit. He reported the development of plasmodia from single spores, but such never fruited, even when placed together, unless brought back to the myxamoeba stage through sclerotization. Skupiński (171) was of the opinion that meiosis occurs during division of the swarm-cells, but offers no cytological evidence to support it. Wilson and Cadman (199) insisted that in *Reticularia* meiosis is completed before the spores are delimited and Cadman (20) reported the same thing

in *Didymium*. Howard (80) found no evidence of a second division in *Physarum polycephalum*.

In spite of this conflicting evidence it is generally agreed that the swarm-cells are haploid and that the nuclei of the mature fructification, with certain exceptions to be noted later, are diploid. It is also agreed that the reduction takes place either shortly before spore formation or is completed in spore germination. The place of nuclear fusion is in somewhat greater doubt. Jahn (89) and Kränzlin (107) believed they found fusion shortly before the final division in the sporangium and the latter author interpreted A. Lister's figures of what he believed to be direct division as in reality stages in nuclear fusion. Later, Jahn (91) found that actual fusion took place between two haploid myxamoebae derived from a single spore, thus inaugurating the diploid plasmodial stage, and that later apparent fusions between such small plasmodia and haploid myxamoebae were not fusions in Cienkowski's sense but that such myxamoebae were devoured by the plasmodium, following which, growth and increase of the diploid nuclei by mitosis occurred. This, of course, did not eliminate the possibility of the anastomosis of small plasmodia, which Jahn observed, and which has since been seen many times. G. Lister (122), after examining Jahn's preparations, concurs in his conclusions. Skupienski (172, 173, 174) reported that in *Didymium difforme* the spores are of two strains, designated as + and -, and that in order to have plasmodium formation it is necessary to have swarm-cells derived from spores of both sorts. Test tube cultures from multi-spored sowing speedily developed visible plasmodia; those in which a single spore was sown developed only swarm-cells and myxamoebae, but two lots of these, when placed together, proceeded to form plasmodia. He found that two to many myxamoebae might fuse in the first stage of plasmodium formation, essentially as reported by Cienkowski. Nuclear fusion did not occur at once, but only after further division. Following fusion, nuclei which had failed to fuse degenerated. Wilson and Cadman found that in *Reticularia* fusion is usually between two swarm-cells and that nuclear fusion follows at once. If, as sometimes happens, fusion is between more than two cells, the nuclei still fuse in pairs, and any that do not find a compatible mate are digested, with their protoplasm. Schünemann (163) studied several species, particularly

Didymium nigripes, observing the development in single spore and multispore cultures. He finds the haploid spores germinate to form haploid swarm-cells, which quickly become myxamoebae and divide repeatedly, at length fusing to form multinucleate plasmodia, nuclear fusion, however, being delayed until there has been considerable plasmodial development. Meiosis precedes spore formation in the sporangium. Cayley (26) cultured several species of *Didymium*, most of her work being done on *D. difforme*. She observed fusion of swarm-cells in this species and concluded that the zygote could develop into a plasmodium but saw no reason to doubt that increase in size may be brought about by coalescence. She succeeded in getting swarm-cells derived from single spore cultures to fuse, and as a result decided that the first meiotic division occurred preceding spore formation and the second in the first division of the swarm-cell. Howard (80) found that in *Physarum polycephalum* fusion was always between swarm-cells, and that nuclear fusion followed almost at once. Cadman (20), studying *D. nigripes*, found that the swarm-cells change to myxamoebae and then fuse. She counted four chromosomes in the swarm-cells and eight in the fusion nucleus, meiosis being completed in the sporangium immediately before spore formation.

Von Stosch (184), studying four species, *Didymium nigripes*, *D. xanthopus*, *D. squamulosum* and *Physarum cinereum*, found nuclear fusion in *D. nigripes* only, the other three species proving to be apogamous. He found the chromosome numbers much higher than those reported by previous workers, that of the triploid apogamous *D. nigripes* about 81. He attributes the smaller numbers reported by others to inadequate fixation. He also reports abundant evidence of amitosis. In the heterothallic form, he finds only one "vegetative" division in the sporangium, meiosis occurring in the spore but only a single nucleus persisting. In the apogamous species the plasmodium is formed by the fusion of numerous amoebae and perhaps also swarm-cells. He also notes that the swarm-cells of a number of species have two flagella, one long and directed forward and another shorter one, difficult to see, appressed to the anterior end, extending earlier observations by others (Vouk, 191; F. A. Gilbert, 59; Howard, 80). Von Stosch's report of apogamy and suggestion that this phenomenon is possibly of widespread occurrence in the group, if confirmed, will be of help in the inter-

pretation of many of the discrepancies in the literature. His results were attacked by Jahn (95) who disputes his account of chromosome numbers, characterizes his report of apogamy as fantastic and regards 2-flagellate swarm-cells as exceptional. Von Stosch's heated reply (185) sheds little light on the differences.

The earlier accounts of the cytology of *Ceratiomyxa* suggested that it was in a class by itself. Famintzin and Woronin (48) regarded the "protospores" from which the stalked "spore" arises as uninucleate. Miller (139) found four nuclei in the mature spore. Olive (141, 142) believed he saw a nuclear fusion just preceding the formation of protospores, and considered the divisions in the spore as meiotic. Jahn (90), on the other hand, believed he found nuclear fusion in the plasmodium before or shortly after it emerges from the wood preparatory to fructification, reduction division taking place before the protospores are delimited, hence the two divisions in the spore do not constitute meiosis. Jahn reported the diploid chromosome number as sixteen. H. C. Gilbert (67) observed the coalescence of swarm-cells, invariably followed directly by nuclear fusion. If this was between two swarm-cells, the nucleus of one remained in place near the base of the flagellum, while that of the other moved through the protoplasm to the position of the former, where fusion occurred. If, as frequently occurred, coalescence was between more than two swarm-cells, half of the fusing nuclei remained in place and the others moved to them, superfluous nuclei being absorbed as in *Reticularia*. Gilbert reported the two divisions in the spore as meiotic, the haploid number of chromosomes being not less than six nor more than eight, in the latter respect agreeing substantially with Jahn. He concludes that the so-called spores of *Ceratiomyxa* are in reality homologous with the sporangia of the Endosporeae, and the sporophores of the former with the hypothallus of the latter, a suggestion made over half a century ago by Zopf (203) but completely disregarded. Gilbert's findings are attacked by both Jahn (95) and von Stosch (185). As Kniep (106) has pointed out, the failure to demonstrate the exact place of meiosis is critical, and additional studies of this phenomenon are urgently needed both with species that have previously been studied and with additional forms.

Closely allied with the cytological studies are the various experiments concerned with so-called sexual phenomena. As found in

the literature, the term sex is used with two meanings. The older and historic concept is that which pictures the individuals of any species as of two sorts, male and female. Even in classical times, the occurrence of bisexuality or hermaphroditism, which has now become a commonplace, was more or less vaguely recognized. Later, maleness was associated with the function of producing male gametes or sperms, and femaleness with the function of producing female gametes or eggs. Associated with this difference, and, of course, recognized much earlier, are the various ancillary devices for bringing about the union of the two gametes. Only with the development of cytology and genetics during the latter part of the nineteenth century was the way prepared for the extension of the term sex to all cases of nuclear fusion whether or not such cases were associated with structures which might be regarded as exhibiting maleness or femaleness. As has been pointed out by several authors in recent years, notably Allen (2) and Link (113), it seems clear that we have to deal with two distinct groups of phenomena. One involves a nuclear cycle characterized by a regular sequence of fusion, reduction and recombination. The other includes the various morphological structures, simple or elaborate as the case may be, whose function it is to produce the sex cells and to bring about opportunity for nuclear fusion under appropriate conditions. Obviously, the former is of primary importance, the latter of secondary significance, but since in all higher organisms, the means has tended to obscure the end, it is not surprising that the terminology has been confused. I venture to suggest that if the term sex and concepts related to it, such as maleness and femaleness, were to be restricted to phenomena of the second group, it would clarify our thinking on the subject. In referring to the more fundamental nuclear processes, the terms karyallagic, and its converse, akaryallagic, proposed by Link, are more precise. Most sexual organisms are karyallagic in this sense, but karyallagy may exist without sex and sex, as in certain water molds, without karyallagy. As so defined, there is no sex in the Myxomycetes but only karyallagy. With this qualification we may examine some experimental studies bearing on the nature of the process.

Abe (1) studied fusion in five species and found that while the swarm-cells functioning as gametes were morphologically similar, the nucleus of one flowed into the other cell and there fused with its

nucleus, exactly as was slightly later and independently reported by H. C. Gilbert for *Ceratiomyxa*. Abe called the cell which retained its nucleus female, and the other, male, and attempted to discover physiological differences between the two. She concluded that the "male" had a higher oxidation-reduction potential than the "female" and that the "female" carries a positive electrical charge while that of the "male" is negative. Kambly (97) attempted to confirm her results, partly repeating her experiments, although with different species, and partly extending them, but was unable to find any evidence for such differences. It is evident that there are differences permitting certain gametes to fuse and inhibiting others from doing so, but it may be assumed that such differences in the Myxomycetes are similar to those permitting fusion between compatible strains in the Basidiomycetes and inhibiting it when the strains are incompatible. In both cases, it seems probable that the distinctions involved lend themselves better to discussion and interpretation if it be inferred that they are in the nature of genetic differences and are not referred to as sexual.

PHYSIOLOGY

The concept of the plasmodium as a naked mass of protoplasm inevitably suggested its use as a source of raw material for analysis and experiment. In the veins, the central mass of granular material, flowing rapidly, with regularly timed pauses and reversal, within an outer mucilaginous sheath, has been observed innumerable times. As early as 1867, Hofmeister had reported positive phototropism and the following year Rosanoff (156) reported the occurrence of negative geotropism in plasmodia. Baranetzki (5) believed that the phototropic response was more important and that the geotropic response might be modified or reversed under the influence of light. Jönsson (96) concluded that what Rosanoff had regarded as geotropism was in reality negative rheotropism. Stahl (181), while recognizing the importance of these three factors, pointed out that others are involved and demonstrated the occurrence of hydrotropism, thermotropism and chemotropism. He emphasized the probable importance of thermotropism to a perenniating plasmodium in spring and fall. Wortmann (202) showed that above a certain critical temperature positive thermotropism is changed to negative. Stange (182) studied the effect of chemicals on swarm-

cells. Clifford (31) confirmed the results of Stahl and Wortmann. Vouk (190, 191) analyzed plasmodial movement and studied the effect of various external factors upon it. He found that light was not necessary for fruiting of the two species of *Didymium*, with white plasmodium, with which he worked. Between 5° and 35° C. Van't Hoff's rule holds; beyond these limits the plasmodium is inactivated. He was unable to discover any influence of gravity, either on direction or speed of protoplasmic movement. In this he is in agreement with Keferstein (99) who claimed that geotropism can not be demonstrated in plasmodia and that rheotaxy has been confused with hydrotaxy. The plasmodia are extremely sensitive to differences in moisture content, including that of air, and while rheotaxy can undoubtedly be shown to exist, it is of subordinate importance. Emoto (44) showed that plasmodia would react positively to weak acids, to most sugars in dilute concentration and to extracts of peptone, meat and various fleshy or subfleshy Basidiomycetes, but reacted negatively to strong acids and alkalis and were indifferent to many other substances, including starch, asparagin and urea. Watanabi, Kodati and Kinoshita (195, 196, 197) and Kinoshita (103, 104) attempted to analyze the movement on the basis of a difference in the electrical charge between the front and rear portions of an advancing plasmodium. They found that this difference is increased by heteroauxins, methylene blue and other substances which increase respiration, while it is decreased by HCN, CO₂ and other substances which check respiration. They conclude that substances which increase respiration raise the potential of the front by stimulating oxidation-reduction activities and that there is a correlation between these changes and the streaming movements.

While the mechanics of this internal motion, as of that of the plasmodium as a whole, are still inadequately explained, there has been no reason to believe that the factors involved in the internal streaming are fundamentally different from those concerned with the streaming of protoplasm commonly seen in the hyphae of fungi and in the cells of green plants. In Myxomycetes, however, it is much more rapid, while the movement of the plasmodium as a whole, at least when on a surface, is similar to that of rhizopods. This is the view of Camp (23). Stiles (183, 432) suggests that in some cases, at least, the protoplasts of a cell enclosed within a cell wall, as in the higher plants, may be capable of amoeboid movements similar to those of the Myxomycetes.

Some of the difficulties apparent in the attempt to interpret the activities of a plasmodium are evidently based on the high capacity for interchangeability of its various parts. This, in turn, may be partially understood if the plasmodium is interpreted, not as a simple structure, but as a highly complex organism singularly well adapted to a protected but none-the-less essentially subaerial life within the interstices of moist, but not sodden, wood, humus or soil.

Various investigators have attempted to analyze plasmodia and fructifications; this work is adequately summarized by Kiesel (102). Lepeschkin's report on the plasmodium of *Fuligo septica* (110) is perhaps the best known. The amount of water is less than might be expected from its consistency, 82.6% of the whole. Of the dry weight, over 40% is composed of water-soluble substances, chiefly carbohydrates and amino acids, and over half of the balance is classed as nucleo-proteins. Other analyses (see, *e.g.*, Kiesel, p. 252, and Stiles, p. 13) do not differ widely. Lepeschkin believed the pigments of the plasmodium were related to the anthocyanins. Solacolu (180) studied the pigments derived from the ripe fructifications of a representative series of twenty-six species and concluded that they differed fundamentally from anthocyanins but were wholly similar to pigments found in the higher fungi. Boić (15) made microchemical tests on the fructifications of a number of species and concluded that the ground substance of the spore membranes is pure cellulose and the wall of the peridium mainly cellulose, but that the capillitium is composed of an unknown substance, possibly protein in nature. Reinke and Rodenwald, cited by Kiesel, seem to have been the first to designate as "plastin" the non-soluble residue left after a plasmodium had been treated with ether, alcohol, water and dilute acids and alkalis. The earlier authors supposed that this was the basic material of the protoplasm, probably distinct in each species. Kiesel's work leads to the conclusion that the plastin is a mixture of two sorts of substances. One group, protein in nature, he would designate by the term myxomycete plastin; the other, which he calls myxoglucosan, in its physical characteristics resembles cellulose and is not improbably the basis for the reports of cellulose as present in spore membranes. Both he would exclude from protoplasm proper, regarding their functions as concerned with the skeleton of the fructification. He points out, properly enough, that much of the work on this subject is based on

plasmodia preparing to fruit, and infers that in an earlier stage the results might be different. Seifriz (166, 167, 168) has repeatedly used plasmodia as material for the study of protoplasm. Lepeschkin had noted a correlation between color and relative acidity of the plasmodium of *Fuligo septica* and Seifriz and Zetzman (170), working with *Physarum polycephalum*, another species with yellow plasmodium, followed the remarkable changes of color exhibited by that form between pH 8 and pH 1.6. Moore (140), using the same species, concluded that an essential part of the living structure is the presence of long threads, which may be as slender as 5×10^{-5} mm. but at least 2000 times as long. Camp (23), also using *Physarum polycephalum*, found no evidence of a sharp distinction between the outer gel and the inner fluid protoplasm, confirming Pfeffer's view (149, 279) that these portions are interconvertible. Kiesel and Camp as well as Jahn (93) add their testimony to that of the earlier workers who insisted that a plasmodium is not the simple mass of naked protoplasm it seems to be at first glance.

The stimuli which induce a plasmodium to fruit are still in some doubt. Klebs (105) found that the plasmodia of *Didymium difforme* and *D. squamulosum* could be maintained in active condition indefinitely by frequent transfer to fresh nutrient material, but that when a small piece of a plasmodium was transferred to a moist substratum without nutrient it soon fruited. Other investigators have suggested, usually on the basis of observation only, desiccation, nature of substratum, light, acidity, injury. Seifriz and Russell (169) discard all these factors, postulating the existence of a rather mystical growth rhythm as a fundamental character of protoplasm. Camp (22), however, like Klebs, found a definite correlation between exhaustion of the food supply and a fruiting stimulus. Gray (70) showed that species with yellow plasmodia require light in order to fruit, while non-pigmented plasmodia fruit equally well in light or darkness. *Physarum polycephalum*, with yellow plasmodium, when placed in lights of various wave lengths, formed sporangia only when exposed to the shorter wave lengths of the visible spectrum. Emoto (47) collected numerous fruitings, representing over a hundred species, from decaying wood and found that in the great majority of cases the substratum was strongly acid, for the most part between pH 4.2 and pH 5.8. Gray (71), in a later paper, reports that in *P. polycephalum*, in addition to light, the factors of

pH and temperature affect fruiting and are interdependent. The higher the temperature, up to 35° C. or somewhat less, the greater the acidity required to permit the formation of sporangia. Gray's studies, while not giving a complete answer to the problems involved, open up what promises to be a fruitful experimental approach and suggest possible interpretations of field observations.

ECOLOGICAL RELATIONS

Many common species of Myxomycetes are among the most ubiquitous of organisms. Even some of the rarer forms, by their widely scattered distribution, display a similar cosmopolitanism. A few species, such as *Trichamphora pezizoides* and *Alwisia bombardea*, are restricted to tropical or subtropical environments, but all such are either known to occur or may reasonably be expected to occur in both hemispheres. R. E. Fries (56), comparing extensive collections from the borders of Bolivia and Argentina with those of Sweden, concludes that the calcareous species are distinctly more abundant in the tropics than the non-calcareous forms, while the reverse is true in the cool temperate regions. This conclusion is in accordance with the reports of Raciborski (154), Penzig (147) and Emoto (43), based on collections in Java, and of Lister (118, 119) on collections from Antigua and Dominica. The African lists of Farquharson and G. Lister (49) and of Duthie (41) and Emoto's Mexican list (45) show an approximately equal division. On the other hand, lists by Jahn (87) from Brazil, by Petch (149) from Ceylon, by F. A. Gilbert (60) from British Guiana and Surinam and by Hagelstein (73) from Puerto Rico show a slight preponderance of non-calcareous forms, while those of Macbride (124) from Nicaragua, Emoto (42) from the Malay Peninsula and Martin (132, 133, 134) from Panamá and Colombia indicate a decided preponderance of non-calcareous species. Obviously a tropical as compared with a temperate climate can not be the deciding factor in such distribution. Carr (24) has shown that in adjacent limestone and sandstone areas in Virginia, calcareous forms are much more abundant in the limestone regions and non-calcareous forms in the acid sandstone areas. This is in accordance with the common experience of collectors, and it seems wholly reasonable to expect such local conditions to affect the distribution of similar forms in the tropics.

A rather specialized group of species occurs in high mountains, appearing just after the snow melts, usually near the margins of snow banks. The ecology of the mountain forms is mentioned incidentally in many taxonomic studies but certain phases of their ecology have been reported by Meylan (136, 137), Macbride (126) and Smith (179). It seems highly probable that the plasmodium remains active under the snow, but this has not been definitely proved. Krzemieniewska (108, 109) secured numerous species of Myxogastres and Acrasieae in cultures from soil and Thom and Raper (188) found at least two species of the Myxogastres and several of the Acrasieae to be abundant in soil and surface litter and conclude that perhaps a considerable proportion of the amoebae reported by soil workers belong in reality to these groups. The common occurrence of *Physarum cinereum* on lawns and of this and related species on crop plants leads to the suspicion that Myxomycetes are common and perhaps significant members of the soil populations. Gilbert and Martin (68) and H. C. Gilbert (66) found a striking group of forms when the bark of living trees was placed in moist chambers, including several supposedly rare or previously undescribed species which proved to be abundant under such conditions.

TAXONOMIC PROBLEMS

The taxonomy of the Myxomycetes is necessarily based almost entirely upon the character of the mature fructification, although G. Lister, in the later editions of the English monograph, emphasizes plasmodial color. The numerous discrepancies in the reports, and the work of Seifriz and Zetzman (170), Kambly (98) and Camp (23) suggest that this character is significant only in the most general way. Every experienced student of the group knows how external conditions may cause variation in size, shape and color of sporangia arising from the same plasmodium. Forms usually sessile may become stalked, and stalked forms sessile, and in the calcareous forms the amount and distribution of lime may be quite different on the upper and lower surfaces of the same log. This variation, which is perhaps the chief cause of the extensive synonymy in the group, while often noted, has rarely been analyzed, largely, no doubt, because of the complexity of the environmental factors involved in the natural fruitings and the difficulty of decid-

ing which of them has had decisive influence. Brandza (16, 17) collected numerous species growing on the roofs of houses in full sunlight and notes marked intensification of color, change of shape and alteration of capillitium. Plasmodia of the same species, transferred to a ravine in a neighboring forest, formed fructifications of the usual type. It is obvious that a detailed knowledge of such possible variations is necessary before species concepts can be regarded as reliable. Cayley (26) and Skupienski (175) showed experimentally that fructifications of various species of *Didymium*, grown in culture, exhibited pronounced variation when the concentration or the pH of the medium was altered. They infer that many characters used in taxonomy may vary in nature as a result of comparable differences in the substratum.

The systems of classification adopted by Lister (117), by Macbride (125) and by Jahn (92), one or the other of which is generally followed today, are highly artificial. Genera, families and orders are based on characters of widely varying significance, as a result of which closely related species are often placed in different genera or families. At the present time, it seems unlikely that study of the plasmodia will throw much light on this subject, although that, of course, is not impossible, but spore germination and morphological development may be expected to do so.

CONCLUSIONS

In the light of our present information, it would seem wise to restrict the term Myxomycetes so that it shall include only the Exosporeae, represented by the single genus *Ceratiomyxa*, and the Myxogastres (Endosporeae). The term Mycetozoa is a synonym, but will doubtless continue to be employed by those who prefer to think of these forms as animals. The Plasmodiophorales constitute a distinct although probably related group, to be placed near or possibly in the lower Chytridiales (Myxochytridiales; Archimycetes *sensu* Gäumann). The Acrasieae are wholly distinct, the pseudoplasmodium characteristic of this group having only a superficial resemblance to a true plasmodium, while the lack of a flagellate stage in the life history constitutes a fundamental difference. The Myxomycetes, as thus defined, can not be regarded as primitive forms, but constitute a specialized group whose affinities are to be sought amongst the colorless flagellates. The Hydromyxales,

while presenting certain suggestive analogies, are also too highly specialized to be recognized as in or very near to the direct line of descent of the Myxomycetes. While the Myxomycetes are by no means so simple as the simplest Phycomycetes, their specialization is of a sort which has led no further. The Phycomycetes, on the other hand, present a reasonably continuous series which, through their higher forms, may well have given rise to the Ascomycetes and, through them, to the Basidiomycetes. The Myxomycetes, then, may be regarded as a distinct class of the fungi, representing the end of one developmental series.

Spore germination is readily secured in the great majority of the species and there is apparently some degree of correlation between details of the process and taxonomic relationships. The products of germination may be myxamoebae or swarm-cells and the number emerging from a spore may, in the Myxogastres, be one to four. The phenomena of germination in a given species are subject to alteration under the influence of environmental conditions and are probably too inconstant to have a bearing on the major problems of classification.

Modern technique has shown that it is possible to culture the plasmodium in large quantities in the case of a few species, and it seems quite probable that there are many other species adaptable to such culture. With proper technique, plasmodia may be grown in pure, *i.e.*, bacteria-free, cultures and under such circumstances their nutrition is saprobic rather than holozoic. It is probable that both types of nutrition are utilized in nature. Once bacteria-free cultures are secured, it should be possible to grow Myxomycetes in pure culture from spore to fructification with no greater difficulty than is entailed in culturing some species of Phycomycetes.

The morphology of many of the larger and better-known species is fairly adequately known, but the life history and cytology of relatively few of them has been followed in detail, and nearly all of these are included in the Physarales. We have an adequate account of but a single species, *Reticularia Lycopodon*, in the pale-spored series. *Fuligo septicola*, *Physarum polycephalum*, *Didymium nigripes*, *D. xanthopus*, *D. difforme* and *D. squamulosum* are mentioned again and again in the literature and constitute the basis for the overwhelming majority of the special studies. A careful examination of one of the large-spored Lamprodermas or Trichias

or of a *Licea* or a species of one of the related genera, should yield significant information. *Ceratiomyxa*, as the only genus of its subclass, has been studied several times, but must be reexamined before the controversy as to its cytology can be settled. There is room for much careful work on the cytology of the fructification, with particular attention to the nuclear divisions immediately preceding spore delimitation.

In view of the grave discrepancies in the literature, it is difficult to present a generalized account of what may be regarded as a typical nuclear cycle in the Myxomycetes, and any attempt to do so must be regarded as highly tentative. It may be accepted, however, that the swarm-cells and myxamoebae are haploid, reduction, in the Endosporeae, being completed either just before the final delimitation of the spores or, less probably, in their germination. After a preliminary period, which may or may not include encystment and division, the swarm-cells or myxamoebae function as gametes, gametogamy taking place between two or more gametes representing two cytologically differentiated strains and karyogamy following immediately or after a short delay, but always, when three or more gametes are concerned, between two nuclei of opposite reaction, superfluous nuclei being merely absorbed as food material. Reports of multiple fusion in which division of haploid nuclei continues after plasmodial formation may possibly be explained on the basis of apogamy, which may reasonably be expected in organisms at this level.

Growth of the zygote into the plasmodium is accomplished by nuclear division and the plasmodium may increase by fusion with others, as is well known to occur in cultures and in all probability is of common occurrence in nature. The conditions which stimulate fructification are not fully determined, but it seems clear that the diploid phase lasts from the time of nuclear fusion at least until the formation of the spores, and that the active haploid phase is brief.

Workers in physiology using plasmodia as an example of protoplasm must be sure the plasmodium is freed of extraneous matter and that it is in an active stage, not preparing to fruit. Recent studies have indicated that it is quite possible to secure plasmodia which meet these requirements.

The current taxonomy of the Myxomycetes is antiquated. Even with our present information, a greatly improved arrangement is possible and should be undertaken.

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PHYSIOLOGIC SPECIALIZATION AND GENETICS OF THE SMUT FUNGI¹

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PHYSIOLOGIC SPECIALIZATION

It has long been known that within species of many fungi there are lines or races that are morphologically indistinguishable but physiologically different. Eriksson (31), in 1894, was the first to demonstrate physiologic specialization in the rust fungi. It was not until 1919 that the phenomenon was recognized in the smut fungi. At that time Kniep (65) reported differences in the appearance of sporidial cultures of *Ustilago violacea* (Pers.) Fuckel from different host plants. In 1921 Zillig (132) demonstrated that physiologic races of this fungus could be differentiated by their ability to infect certain members of the Caryophyllaceae but not others.

The term physiologic race has been used to designate groups within species that differ in one or more of the following characters: pathogenicity, cultural characters on artificial media, physiologic and ecologic characters, biochemical effects, and morphology. It is generally recognized that a physiologic race, as the term is used in connection with the smut fungi, is not strictly comparable to races within species of rusts and certain other fungi. The term physiologic race is used in the Ustilaginaceae to designate a collection of chlamydospores that behaves more or less consistently in parasitism on certain differential varieties of host plants and not others. As pointed out by Holton (47) and Churchward (21), the

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use of the term in this connection is not strictly correct. The chlamydospores represent the diploid phase, which can not be propagated independently. When they germinate, reduction and segregation of various factors take place and there must be fusion between haploid lines of opposite sex before infection of the host plant can occur and chlamydospores again be formed. Consequently, each new generation of chlamydospores may consist of a new group of related biotypes. In another sense the term physiologic race in the smut fungi has been applied to haploid lines that differ in growth characters on artificial media. Since the haploids alone can not cause infection, they should probably more accurately be referred to as lines or biotypes. For utilitarian purposes it has seemed desirable to retain the use of the term physiologic race in the smut fungi for collections of chlamydospores having the same relative virulence on certain differential varieties.

*Physiologic Specialization in Relation to Breeding for Smut
Resistance*

It has been pointed out repeatedly that no other factor has contributed more to the difficulty of obtaining smut-resistant varieties of cereals than physiologic specialization in the pathogens. Prior to the time that information on the prevalence of races was available, the production of varieties resistant to smut appeared to be a relatively simple problem. For example, in 1922, Stephens and Woolman (114) listed twenty varieties and selections of wheat that seemed safe for sowing without seed treatment. Likewise, Schafer, Gaines and Barbee (101) reported that the wheat varieties Albit, Hussar, Martin, and Ridit were all highly resistant to bunt, but all are now known to be susceptible to one or more races of *Tilletia* (94).

In most instances the outbreaks of smut in supposedly resistant varieties have been definitely associated with the appearance of previously undescribed races. For example, until 1925 the durum grown in the Hard Red Spring wheat area were classed as bunt-resistant. About that time, however, they became rather generally smutted. In pathogenicity tests, Holton (47) found collections of this smut to represent a previously unidentified race, characterized by its marked ability to attack severely the durum wheats. Likewise, in connection with the oat smut pathogens, Reed and Stanton (88) found increased susceptibility in the so-called resistant

Fulghum and Red Rustproof types of oats to be definitely associated with two previously undescribed races of *Ustilago avenae* (Pers.) Jens. To prevent such disappointments and insure greater success in the program of breeding for smut resistance, data on the number, prevalence, distribution and virulence of races are being accumulated (24, 32, 37, 39, 79, 87, 89, 94, 96, 97, 115, 131).

In testing for smut resistance it is now generally the practice in preliminary experiments to inoculate seed with a composite of chlamydospores collected throughout the area to which the varieties are adapted. Susceptible lines may then be discarded. As shown by Holton and Heald (54), however, the use of composites of a large number of collections may not be a reliable method of determining absolute resistance of varieties. To determine the true index of resistance, supplementary tests should be made in which desirable lines selected in the preliminary experiment are inoculated separately with each of the known races. Failure to do this may result in a repetition of the experience encountered with the Hard Red Winter wheat Yogo (C. I. 8033). In bunt nursery tests where composite inoculum was used, this variety was repeatedly classed as resistant and was therefore released for commercial production in 1935. That year Yogo, among other varieties, was inoculated with individual races and found to be highly susceptible to a race now known to be generally distributed in the area to which this variety is adapted.

Difficulties also may be encountered in obtaining accurate records of the resistance and susceptibility of varieties to smut because of differences in response of both parasite and host to different environmental conditions. This is particularly true as regards the bunt pathogens and their host varieties. Aamodt (1) has pointed out that when subjected to different temperatures some physiologic races appear to respond differently in infection capability. Rodenhiser and Holton (94) found the variety Turkey (C. I. 6175) to differ in susceptibility to certain races when grown under different environmental conditions. This variation appeared to be due to the effect of environment on the host rather than on the parasite, for the variety Hybrid 128 (C. I. 4512) remained uniformly susceptible to the same races in comparable tests. Likewise, the spring wheat variety Marquis has maintained its resistance to bunt at University Farm, St. Paul, Minnesota, for a great many years, but it is com-

pletely susceptible to the same race of *Tilletia* at Bozeman, Montana. Here again it probably is the effect of environment on the host plant because *Ceres* has been recorded as susceptible to the same race in comparable tests at the two stations. There also is evidence that the reaction of a variety of wheat to bunt may be influenced by the environmental conditions to which it is exposed during the previous growing season (46, 54). When Marquis wheat from Pullman, Washington, was grown for one year in another locality, returned and tested at Pullman, Holton and Heald (54) found differences in susceptibility to certain collections of both *Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kühn. In their experiments the lots grown at Laramie, Wyoming, and Logan, Utah, in general had the lower percentages of bunt, while the lot grown at Fargo, North Dakota, for one season produced the maximum amount. These differences in response of the parasite and the host to different environmental conditions emphasize the importance of subjecting those progenies selected for smut resistance to smut tests made under a rather wide range of environmental conditions.

Comparisons of data on smut tests made at different stations and by different investigators are frequently made, and where variability in the smut reactions of any one variety occurs it is usually assumed that there was a difference in the races used or that the environmental influences were not the same. However, such differences also may be due to the use of different strains or selections within a variety, any one of which may react differently to one or more races. For example, Heald (46) tested, under comparable conditions, seed lots of Turkey Red from seven different regions and found the percentage of smutted plants to vary from approximately 14 to 65. Likewise, Holton and Heald (54) tested different selections or seed lots of Marquis from seven regions, and the percentage of smutted plants varied from 18 in the Washington Marquis to a maximum of 43 in a Minnesota strain. The extent to which two selections may vary in susceptibility to different races of *Tilletia* is emphasized in tests with Turkey (C. I. 11530) and Regal (C. I. 7364). Although sister selections, the former has the Hussar type and the latter the Albit type of reaction to the known races recently described (94).

The present understanding of many of the factors that contributed to the difficulties in obtaining smut-resistant varieties

should help materially in the solution of the problem. More information is available concerning the number, prevalence, and distribution of races; and, furthermore, a number of varieties and selections are now known to have factors for resistance to many of these races that are rather widely distributed. Thus, the plant breeder may more intelligently select parental material to be used in crosses and can recognize the limits within which his efforts are likely to be successful. As an example, Vogel and Holton (124) have utilized this information in the production of the smut-resistant wheat, Oro \times Turkey—Florence. Oro has factors for resistance to all of the known races except L-8, and Turkey \times Florence has resistance to all except T-11. By combining these factors for resistance, certain progeny were obtained that are resistant to all of the known races of *Tilletia* and which also appear to possess many of the desirable agronomic characters of both parents. Similarly, in a definitely planned hybridization program, Coffman *et al.* (22) reported that they have transferred the Markton factors for resistance to the oat smut pathogens to certain progenies of Markton \times Rainbow crosses. Although it has been possible to produce these hybrids with resistance to the known races of the pathogens, breeders are not failing to recognize that the problem may be still more or less a changing one for some time to come. Some of the cardinal races probably have not yet been identified and there is always the possibility of new races appearing as a result of hybridization, mutation, or introduction from another geographic area. While hybridization and mutation probably occur commonly under natural conditions, it must be remembered that the chances of survival of new races thus formed are not particularly great. If new races are to persist, the environmental conditions must be favorable, susceptible host plants must be present, and there must be opportunity for multiplication and dissemination. Breeders also recognize that changes may occur in the host varieties. Regardless of what the explanation may be, it is frequently found that, soon after the distribution of new varieties, selections may be made which differ in yield and other factors from the original stock. For example, Stadler and Kirkpatrick (105) pointed out that although the Fulghum variety of oats is supposed to have originated as an individual plant selection, 25 years later all commercial stocks of the variety consisted of a mixture of strains varying in productivity and to some extent in visible char-

acteristics. Obviously, factors responsible for such genotypic changes in the host may also affect changes in the reaction of the smut pathogen.

Selective Effect of Host on Pathogenicity

The question often has been raised as to whether increases in virulence of the bunt fungi on certain wheat varieties is due to adaptation of the fungus or to the "screening" effect from collections of chlamydospores comprising more than one race. In this connection, Dillon Weston (29) and Bressman (16) reported increases in the percentages of bunt on the variety Ridit by inoculating seed of this variety with bunt obtained from infected Ridit plants. Melchers (75) used the same technic and obtained increases in percentages of infection on White Odessa and Hussar. It has been pointed out by Flor (37) that because of the limited knowledge concerning the genetics of the bunt fungi and the inability to know whether the collections of bunt contain one or more races, it is debatable whether the apparent change in virulence is due to the adaptation of the fungus to the host or to a screening out process whereby only those races in the collection are propagated that are able to attack the resistant host normally. Flor (37) studied two collections of *Tilletia* with reference to the screening effect of the host variety on the percentage of bunt in the same variety and in certain other varieties in the subsequent crop. From one collection two pathogenically distinct races were isolated: One was a race of *Tilletia levis* to which Oro was completely susceptible and to which Albit, Hussar, and White Odessa were resistant; the second was a race of *Tilletia tritici* to which Oro was resistant and to which Albit, Hussar and White Odessa were susceptible. His results with a second collection indicated that its pathogenicity had not been changed by adaptation or by the "screening" effect of the host. Bever (11) recently reported no significant increase in percentage of bunt infection when resistant varieties were reinoculated with their own smut over a three-year-period. Roemer and Kamlah (95) studied the screening effect of the host on *Ustilago tritici* and found the virulence of the fungus to be greatly influenced by the selective influence of the host. Likewise, Reed (86), Nicolaisen (80, 81) and Vaughan (123) obtained evidence of screening effects with the oat smut pathogen, as did Sampson and Western (99)

with *U. avenae* and *U. kollerii* Wille (*U. levis* (K. and S.) Magn.). The latter investigators studied the behavior of six collections of oat smuts over a ten-year period and found changes in pathogenicity to have taken place in three of the collections. It is concluded from these experiments that the change in virulence is due to a screening process taking place in the host by the selection of biotypes from chlamydospores which are heterozygous for factors governing pathogenicity. Western (130) has pointed out that most frequently under natural conditions the invading parasitic mycelium is derived from the promycelium which is the immediate result of germination of the chlamydospore. This being the case, as suggested by Sampson and Western (99), it is possible, theoretically, for the heterozygous condition to persist through a number of chlamydospore generations.

The persistence of the assumed heterozygous condition presents a disturbing factor in the interpretation of results regarding the stability of pathogenically distinct races. It is evident from studies on artificial media and from the appearance and behavior of phenotypes in the field that the smut fungi are extremely heterozygous for many characters, and consequently unstable. On the other hand, races of the smut fungi have appeared to be relatively stable as regards factors for pathogenicity. Reed (87), for example, has pointed out that the Missouri races of loose and covered smuts of oats have been grown annually for more than 20 years, and throughout that period of time have shown a constant behavior on given varieties of oats. Likewise, Flor (37) and Rodenhiser and Holton (94) have found certain races of the bunt fungi to be remarkably stable over a period of years. In view of the possibility of the persistence of factors for pathogenicity in a heterozygous condition, generalizations should not be made too freely as regards the stability of these factors.

STUDIES ON GENETICS OF SMUTS

Mechanism of Heredity

The smut fungi, particularly those that commonly produce sporidia, are especially suitable for genetic studies. The individual sporidia, which are usually uninucleate and unisexual, can be isolated easily and propagated on artificial culture media (16, 23, 41, 68, 74, 110). The progeny of a single sporidium, barring muta-

tion, constitutes a clonal line; hence they are genetically alike. The sporidia of most species of smuts in the Ustilaginaceae bud rapidly in a yeast-like manner so that a colony 7 to 10 days old growing on a favorable nutrient medium may contain millions or even billions of unicellular and haploid individuals. Hence the behavior of a colony of smut involves a very large population of potential gametic lines grown under very similar conditions. A splendid opportunity for the study of mutation and environmental modifications is offered by haploid lines, because they can be subjected to conditions more extreme than higher plants and animals can tolerate; they also can be exposed to conditions which are carefully controlled, thus making possible the detection of minor mutations. The nature of change can be determined by breeding behavior. In some species many chlamydospore generations are possible in a single year. Furthermore, haploid lines usually can not cause infection and the fusion of two lines of opposite sex is a prerequisite to normal infection. This makes possible genetic studies of dicaryophytes, especially their parasitic behavior which results from nuclear association. Moreover, interspecific and intergeneric hybridization occurs and a study of the resultant progenies, even under very precise conditions, is relatively easy.

Nature of Chlamydospore Germination. Mature chlamydospores of the smut fungi, with a few possible exceptions, are diploid. On germination, the chlamydospores of many of the smuts produce a promycelium in which reduction and segregation normally occur. In the Ustilaginaceae after the promycelium has grown to about one third of its length the diploid nucleus migrates into it and divides; in those cases where meiosis occurs in the chlamydospore, either one or both haploid nuclei migrate into the promycelium. Typically three septa are formed in the promycelium, although marked variation in septation is not uncommon, especially in certain species or crosses. Usually a single sporidium or hyphal branch is produced from each promycelial cell, but sometimes two or more are developed. The promycelial nuclei do not pass out into the sporidia but divide, and each of the daughter nuclei enters a sporidium. This nuclear process may be repeated several times, and as a consequence, it is possible, as in *Ustilago zae* (Beckm.) Ung. and *Sphacelotheca sorghi* (Lk.) Clint., to obtain several crops of sporidia directly from a promycelium, which makes possible a

genetic study of sporidia budded successively from the same promycelial cell (20, 49, 103). In no other group of fungi can such a genetic analysis be made of successive nuclear division during meiosis. In the Tilletiaceae, the nuclei usually pass into the sporidia or hyphal branches, none remaining in the promycelium (50, 82, 111).

The range of variation in the type of germination of chlamydospores is of the utmost importance, especially when a genetic analysis is to be made of progenies from individual spores. In many species of smut much irregularity may be found in the number of septa and primary sporidia formed. For instance, it is not uncommon to find a promycelium with a single cross wall giving rise to 2 to 4 primary sporidia from the upper segment and several more at the junction of the promycelium and chlamydospore (20, 92). Occasionally chlamydospores develop two promycelia (15, 20). Sometimes the germinating chlamydospores of certain smut fungi that normally produce sporidia develop hyphae directly from the promycelium (2, 64, 70, 125, 130).

The type of germination depends upon the genetic constitution of the spores and upon the environmental factors (temperature, moisture, light, oxygen, *etc.*) at the time of germination (20, 58, 59, 61, 64, 106, 113, 116). Allison (2) noted that chlamydospores of *Ustilago hordei* (Pers.) K. and S. which germinated beneath the hulls of barley often produced no sporidia but only promycelial segments which fused (knee joints) and gave rise to infection hyphae. Western (130) obtained similar results with *Ustilago avenae* while Walter (125) noted that infection in corn plants may take place by the penetration of the promycelium of *Ustilago zeae*. Obviously, direct infection from promycelia minimizes the opportunity for genetic variation. Hüttig (55) found that a low temperature frequently induced sporidial production even in species that normally produced no sporidia, as in *Ustilago nuda* (Jens.) K. and S.; while under high temperature *Ustilago longissima* (Sow.) Tul. had a tendency to produce hypha-like growths instead of sporidia. De la Camp (25) and Thren (117), however, could not induce sporidial formation in *Ustilago tritici* (Pers.) Rost. and *U. nuda* by germinating the spores at low temperatures, although promycelial cells separated under these conditions.

Meiosis. Extensive studies show that the meiotic division normally occurs in the chlamydospore or in the promycelium. Reduc-

tion in chromosome number usually takes place in the first or second division of the diploid nucleus, but apparently it also may occur in later divisions. In a few cases seemingly no reduction in chromosome number or segregation for sex factors occurs until the next chlamydospore generation (17, 20). In fact, Christensen (20) found in *Ustilago zaeae* no reduction of factors for sex in three successive chlamydospore generations. The sporidia were diploid (solopathogenic) and capable of causing infection and forming chlamydospores when inoculated into the host.

Hanna (41) and others (27, 49, 92, 118) have suggested on the basis of their segregation studies that one pair of chromosomes may reduce in the first division and another pair in the second division. Some of their data might be explained on the basis of crossing-over at the double strand stage in the first division of the fusion nucleus, and subsequent segregation of factors in the second division. At present, certain data on segregation of factors for sex and cultural characters can not be explained satisfactorily on the basis of crossing-over without assuming reduction in the second division. If reduction of chromosomes occurs commonly in the second division, one might expect frequent segregation in the third division due to crossing-over. Much more information is needed in regard to crossing-over in smut fungi.

Hüttig (55), Popp and Hanna (83) and others (2, 20, 28) found segregation of factors for sex occurred predominantly in the second division, while Miss Wang (126) concluded that, in *Tilletia tritici* and in all *Ustilago* spp. which she studied cytologically, reduction in number of chromosomes always occurred in the first division. This would mean, if she is right, that crossing-over must be extremely common to account for the apparent segregation of factors for sex and for cultural characters on the same promycelium. Studies with *Ustilago zaeae* indicate that reduction for cultural characters may occur in the promycelium without segregation for sex. Thus, Christensen found that a set of monosporidial lines of *U. zaeae* were all solopathogenic, yet they were distinctly different culturally (20). Holton's (49) and Dickinson's (28) studies on inheritance in the smuts of oats indicate that reduction and segregation may extend beyond the second division of the fusion nucleus. In *U. zaeae*, Stakman (107) and Christensen (20) obtained two different haploid lines from the same promycelial cell and in *Spha-*

celotheca sorghi Tyler (118) obtained two different haploid lines from the same promycelial cell. However, in certain crosses, Stakman (107) found individual segments of the promycelium with several nuclei. Consequently, what might appear as delayed reduction was in reality due to nuclear irregularity. Although Stakman did obtain indication of delayed segregation in abjoined sporidia, he concludes that, in general, segregation in *U. zae* was complete in the promycelium (107).

Solopathogenic lines of *Ustilago zae* or monosporidial selections from them occasionally become non-pathogenic (19, 20). Numerous attempts were made to combine these lines with haploid testers for sex, but all lines, except two, gave negative results.³ Repeated tests with these two originally solopathogenic lines indicate that the sporidia became haploid after being grown in culture media for many asexual generations. Whether these two haploid lines arose from a diploid nucleus by means of segregation or mutation, however, was not determined.

There is some evidence that the onset and duration of meiosis as determined by nuclear division may be influenced by environmental conditions at the time of chlamydospore germination. Hüttig (55, 56) showed that temperature and certain salts influenced the time of reduction, while osmotic pressure and hydrogen-ion concentration did not. Bauch (7) and Dickinson (28) have shown that temperature may modify the proportion of reduction in first or second division. In general, high temperature had a tendency to increase the frequency of reduction in the second division. Dickinson (28) concluded that in *Ustilago levis* the process of meiosis may be affected by the hydrogen-ion concentration of the medium and by the relative proportions and total concentration of carbohydrates and nitrogen in the medium. From these studies he concluded that segregation for various cultural factors, but not factors for sex, might occur in the third nuclear division. Moreover, reduction and segregation might occur in two non-consecutive divisions. Numerous attempts have been made by Christensen⁴ to inhibit reduction of factors for sex in *Ustilago zae*. Chlamydospores derived from various sources, especially those derived from solopathogenic lines of *Ustilago zae*, were germinated on many types of nutrient media and at different temperatures. Subsequently, numerous mono-

³ Unpublished data by J. J. Christensen.

⁴ Unpublished results.

sporidial lines were isolated at random but all were unisexual. It seems likely that the environmental factor that influences the time of reduction varies with the species, the physiologic race, and with the particular cross. Chilton (17) found that the formation of solopathogenic lines in *U. zae* is associated with lethal factors producing lysis of promycelia and sporidia.

Chromosome Number. Much of the evidence for reduction and segregation is circumstantial and is obtained by the growing of sets of monosporidial progenies from individual chlamydospores, sporidial or promycelial fusions, and by making pathogenicity tests. However, this is supported by a certain amount of cytological evidence. There are no inheritance studies that indicate the number of chromosomes present in smut fungi.

The diploid chromosome number in most of the species of smut fungi studied is four. Rawitscher (85) reported four for *Tilletia tritici*, while Kharbusch (62) found four in *Ustilago hordei*, *U. hypodytis* (Schlecht.) Fries, and *U. zae*. Hüttig (55) found four in *U. avenae* and *U. hordei*. Miss Wang (126) studied nine species of smuts belonging to three genera, *Ustilago*, *Tilletia*, *Sphacelotheca*, and all had four chromosomes in the diploid stage. In 1898 Harper (45) reported from 8 to 10 chromosomes in *Ustilago scabiosae* Sow. If this is the normal number of chromosomes for this species, it should prove a most interesting fungus for cytological and genetical studies.

At present relatively little is known concerning the chromosome behavior of the smut fungi. The nuclei are extremely small (from $1\frac{1}{2}$ to $3\ \mu$ in diameter) and most workers find it difficult to observe the early prophase and to make preparations in which chromosomes are sharply defined. The best results have been obtained by staining the chromosomes in the promycelium and in the chlamydospores just prior to germination.

Origin of the Dicaryophyte. Some smut fungi are homothallic while others probably may complete their life cycle in a seemingly haploid condition. For instance, Boss (13) gives *Ustilago ischaemi* Fuckel as an example of a smut in which no nuclear fusion occurs. Blizzard (12) concluded that the parasitic mycelium of *Urocystis cepulae* Frost. remains uninucleate until just before sporulation. Fleroff (35) claims that a uninucleate strain of *Ustilago avenae* produced chlamydospores on oats. There is, of course, a possibility

that this smut was diploid instead of haploid. Christensen (19), Eddins (30), Sleumer (104) and Chilton (17) found monosporidial lines of *Ustilago zeae* that caused infection when inoculated singly into the host. Extensive studies (20) indicate that the sporidia of solopathogenic lines are usually bisexual. Since they are uninucleate they must be diploid, because the sporidial progenies of chlamydospores developed from solopathogenic lines are usually haploid. In *Urocystis waldsteiniae* Pk., Hanson and Atkinson (44) found that the haplophase had been almost eliminated. Following meiosis in the chlamydospore the young promycelium and all subsequent vegetative development was in the dicaryotic condition.

Although it has long been known (33, 45, 68) that fusions between sporidia occurred commonly, it was not until 1919 that Kniep (65) demonstrated that fusion took place only between sporidia of opposite sex. Kniep (69) first explained this type of fusion on the basis of genetic factors for sex, but later changed it to factors for copulation.

After two sporidia of opposite sex fuse the protoplasm and nucleus from one sporidium usually pass through a fusion tube into the other sporidium, or else both nuclei enter the fusion tube. Shortly after the association of the two nuclei, an aerial hypha designated by Bauch as "Suchfaden" is developed from the binucleate sporidium or more commonly from the fusion tube between the two sporidia. In smuts in which no sporidia are formed, fusion may occur between two adjacent or non-adjacent hyphal segments of opposite sex of the same promycelium or between two segments of different promycelia by means of a fusion tube (67, 73).

Some mycologists consider that the dicaryophase is essentially equivalent to the diplophase because the association of the two genetically different nuclei clearly influence the morphology, physiology and pathogenicity of the hyphae (49, 68, 79). Haploid hyphae are usually rather fine and seldom cause infection, being unable to grow extensively in the tissues except in a few cases (12, 13). Dicaryotic hyphae, on the other hand, are large, usually grow more vigorously, and are parasitic (2, 69). It is noteworthy that diploid lines of *U. zeae* (solopathogenic lines) in culture behave in a manner similar to haploid lines, while in the host they behave as dicaryophytes (19, 20).

Fusion of haploid sporidia or promycelial cells is only the first

step in hybridization—a plasma hybrid. It is an act of cell fusion and association of nuclei, not necessarily resulting in fertilization (syngamy), but rather in the initiation of the dicaryophyte. Two haploid nuclei with opposite sexual tendency become associated through plasmogamy, but the dicaryons may lack the necessary factors for parasitism or the nuclei may lack ability to fuse, presumably possessing insufficient sexual attraction (2, 7, 8, 77). Consequently, there seems to be considerable justification for restricting the term diplophase to the chlamydospore and in the young promycelium in those cases where meiosis has not yet taken place.

There is a tendency for the nuclei of dicaryons to dissociate in culture and in the host. Several investigators (13, 26) have shown that dicaryotic mycelium in culture may bud off unisexual and uninucleate sporidia. In fact it is difficult to maintain in culture the dicaryotic stage of those smut fungi which are normally heterothallic. As a consequence, genetic study of dicaryons of most smut fungi can not be made on nutrient media but must be made in the living host. Obviously, this greatly hinders certain inheritance studies that might otherwise easily be made. Dissociation of genetically different nuclei apparently occurs even in the host, as monosporidial lines of *Ustilago zaeae* isolated from parasitic mycelium and young galls derived from the union of two haploid lines in the corn plant are always haploid, while those that originate from a solopathogenic line are always diploid (20, 41). The reisolated lines are identical culturally to the lines used in making the original pairing, indicating that mixing of cytoplasm has no apparent effect on the asexual progeny (41).

Chlamydospore Formation. Many workers (12, 23, 45, 68, 73, 84, 111) have studied the nature of chlamydospore formation in the host, and there is a general agreement that the young chlamydospore is binucleate but on maturity has a single diploid nucleus. Chlamydospores arise from transformed dicaryotic cells of parasitic hyphae. The method by which the transformation occurs and by which the two haploid nuclei become associated in the spore varies with the species. The mycelium in the host is usually binucleate, but it also may be uninucleate or multinucleate. In some smut fungi, clamp connections are common; in others, their presence has been questioned, and in still others none has been observed (98, 102, 104, 113). Although the method by which two nuclei of opposite

sex become associated in pairs in the cell or segment varies, karyogamy usually occurs immediately before the cell is transformed into a mature chlamydospore.

Chlamydospore formation in culture has been reported for many species of smut fungi, but in many cases there is no evidence that they are diploid and can germinate by the production of a typical promycelium (15, 40, 66, 71, 126, 128). Until recently, very little attention has been paid to nuclear behavior during the formation and germination of chlamydospores produced on artificial nutrients.

In 1919, however, Fleroff (34) germinated chlamydospores of *Ustilago hordei* produced in culture. He found that they germinated normally, but in a later publication he (35) states that chlamydospores of *U. avenae* developed in culture were uninucleate and fusion of nuclei did not occur. Boss (13) also concluded that chlamydospores of *Tilletia tritici* and *Ustilago ischaemi* produced in culture were haploid. Stempell (113) obtained uninucleate chlamydospores from monoconidial lines of *Entyloma* spp. Other investigators have observed nuclear fusion in chlamydospores produced on artificial culture. Sartoris (100) states that chlamydospores of *Ustilago heufleri* Fuckel produced in culture behave like chlamydospores developed in the host. Wang (127) found that chlamydospores of *U. crameri* Koern. produced on sterile media were of the same size and structure as those produced in the host, and they germinated in a normal manner. No inheritance study has been made of chlamydospores formed in culture. If chlamydospores could be produced readily on artificial media and induced to germinate freely, the genetic study of many smuts would be greatly simplified.

Determination of Sex Groups. In genetic studies of the smut fungi it is important to know the sexual compatibilities of monosporidial lines or hyphal segments, since the pairing of lines of opposite sex in most smuts is normally prerequisite to infection. Several methods of determining sexual compatibility have been reported. In the smut fungi that produce sporidia, sporidial fusion is usually used as the sex index, while in those which germinate by means of a promycelium only, fusion between segments may serve as a criterion. Bauch (9) found that the type of growth of colonies of sexually compatible paired lines of *Ustilago violacea*, *Sphacelotheca schweinfurthiana* (Thum.) Sacc., *U. scorzonerae* (Alb. and

Sch.) Schraet., and *U. zeae* was distinctly different from that of monosporidial lines or sexually incompatible paired lines, and he considered this a reliable criterion for determining sex. However, fusions are not readily detected in *U. zeae* and in certain other cereal smut fungi, nor has the Bauch test proved reliable in all cases. Inoculation tests with the subsequent formation of chlamydospores from compatible lines is therefore more generally used. The Bauch test has been satisfactorily used in determining sex groups in *S. sorghi*, *S. cruenta* (Kühn) Potter and some other smuts, particularly when sporidial lines are used, but it is unreliable for *Ustilago levis* and *U. avenae* (49). Additional criteria for the determination of sex groupings have been reported for *U. zeae*, *S. sorghi* and *S. cruenta*. When paired monosporidial lines of *U. zeae* are injected into certain varieties of corn, only those that are sexually compatible stimulate the production of anthocyan (20, 41). Likewise, only sexually compatible paired lines of *S. sorghi* and *S. cruenta* cause chlorotic flecking when injected into sorghum plants (91, 118).

There is some evidence that sexual groups may respond quite differently to nutrients. Bauch (3, 6) found that colonies of *Ustilago violacea* belonging to one sexual group were suppressed on certain cultural media, while colonies of the opposite sex thrived. Thren (117) noted a somewhat similar relationship between (+) and (-) lines of *U. nuda*, although the relationship was not perfect. Moreover, such an association could not exist in species with 3 or more sexual groups.

Inheritance of Specific Characters

Because of the simplicity of the sexual processes involved in the smut fungi it has been relatively easy to demonstrate hybridization in the organisms. In those that produce sporidia, haploid lines are isolated and those of opposite sex paired. When paired, fusions occur between the lines, and the two haploid nuclei become paired in the fusion tube or resulting infection hypha. These nuclei remain associated throughout the period of growth of the mycelium in the host plant and fuse at the time of chlamydospore formation. The diploid nucleus in the mature chlamydospore thus contains hereditary factors common to both parent lines.

Size of Chlamydospores. The size of chlamydospores may vary considerably with the race of smut within a given species (20, 50,

72, 76) and this character is inherited. Kammerling (60) found that the F_1 chlamydospores between *Ustilago longissima* and *U. longissima* var. *macrospora* Davis were intermediate in size. There was no indication of dominance of factors for size as all F_1 chlamydospores tend to approach the averages of the two parental types. Tyler and Shumway (119) found this to be true in crosses between *Sorosporium reilianum* (Kühn) McAlpine and *Sphacelotheca sorghi*, and Vaheeduddin obtained similar results with *S. reilianum* and *S. cruenta*. Allison (2) obtained similar results for crosses between *Ustilago hordei* and *U. medians* Biedenkopf. The number of genetic factors that determine the size of chlamydospores is not known, but probably more than one set of factors is involved.

Wall Markings. Several workers have studied the inheritance of spore-wall markings in smuts and have concluded that they are inherited in a simple manner. Holton (48) and Popp and Hanna (83) found that F_1 chlamydospores between *Ustilago avenae* (spores echinulate) and *U. levis* (spores smooth) were echinulate, indicating dominance for echinulation. In the F_2 population the echinulate and smooth chlamydospores were distributed on a 3:1 basis, indicating a single factor difference (52).

Allison (2) and Moore and Allison (78) found that F_1 chlamydospores in all interspecific crosses between *Ustilago hordei* (spores smooth) and *U. medians* (spores echinulate) were echinulate, like those of *U. medians*. Tyler and Shumway (119) noted that the F_1 chlamydospores between *Sorosporium reilianum* (spores echinulate) and *Sphacelotheca sorghi* (spores smooth) were echinulate, while Rodenhiser (93) noted the same for F_1 hybrid chlamydospores of *S. sorghi* and *S. cruenta* (spores inconspicuously echinulate). These segregation studies indicated that echinulation is determined by a single pair of factors.

Flor (36) and Hanna (42) state that hybrid chlamydospores from crosses between *Tilletia levis* (spores smooth) and *T. tritici* (spores reticulate) were all like those of *T. levis* (spores smooth). Recently, Holton (53) found smoothness of wall in this interspecific cross to be recessive. Since there are varying degrees of spore markings within a species, one may expect more than one set of factors are involved.

Spore Color. The study of inheritance of chlamydospore color has been made possible by the existence of a buff race of oat smut

and by an albino race of *Ustilago hordei* (48, 78). Holton (52) showed that the factor for brown color in chlamydospores of the oat smuts was dominant over the factor for hyaline chlamydospores and that distribution of the F_2 population was on a simple 3:1 basis.

In a cross between *Ustilago avenae* (spores brown and echinulate) and a buff race (spores hyaline and smooth) the chlamydospores were brown and echinulate, two pairs of factors for echinulation and for color being involved. Consequently, one would expect the dihybrid ratio 9:3:3:1. However, the F_2 population of chlamydospores was distributed into classes giving a 9:3:4 ratio: echinulate brown, smooth and brown, smooth and hyaline, respectively. There were no hyaline echinulate chlamydospores. The true cause for lack of expression of echinulation in hyaline chlamydospores is not known. Perhaps the epispore has been lost.

Type of Smut. Genetic factors govern the general morphology and the consistency of the smut sori, the color of peridia, and the degree to which the host plant may be stunted (50, 90, 92, 118). Since the hyphae that produce these characters are in the dicaryotic condition, one might expect the smutted heads on the same variety to be alike, but this is not always the case. Holton (49) found that inoculation with compatible monosporidial lines of *Ustilago levis* gave a covered type of smut, while crosses between lines of *U. avenae* produced loose, covered, or an intergrading type of smut. The type of smutty panicles produced in certain crosses depends on the interaction of the host and the fungus to a particular environment; hence it is not surprising that crosses between *U. levis* and *U. avenae* produced loose, covered, and intergrading types of smut. Both the loose and covered type of smut may be produced on the same variety of oats by the same dicaryophyte, and the same dicaryophyte may produce loose smut on one variety and covered smut on other varieties (49).

In crosses between *Ustilago hordei* (covered type) and *U. medians* (loose type), Allison (2) found that the F_1 head types were intermediate, tending somewhat toward the loose head type. In the F_2 generation, besides the parental types, various intergrading types also appeared; consequently two or more factors determine the head type. Incidentally, the chlamydospores were echinulate in some of the intermediate types and smooth in others, as in *U. hordei*. Rodenhiser (92) found that F_1 sori of interspecific

crosses between *Sphacelotheca sorghi* (covered type) and *S. cruenta* (loose type) were characteristic of the loose smut. Later he found the progeny of a backcross to segregate in an approximate 1:1 ratio for smooth and echinulate chlamydospores (93). Vaheeduddin (121) found that different monosporidial combinations of an intergeneric cross between *Sorosporium reilianum* (head smut) and *S. cruenta* (loose kernel smut) produced sori differing strikingly in size and shape. Some sori resembled those of head smut, some the loose kernel smut, and others were intermediate in type. In fact, all gradations were encountered; and in one cross the sori were quite similar to those of long smut (*Tolyposporium filiferum* Busse). There were great differences in dominance, depending on the particular lines used in the cross. Apparently smut types in these crosses also were governed by several factors.

Studies on the inheritance of peridial color indicate that the factors for this character are not always inherited on a simple Mendelian basis. Rodenhiser (92) found that in certain crosses between monosporidial lines of *Sphacelotheca sorghi*, the reddish brown color of the peridium was dominant in F_1 , while in *S. cruenta* the gray color was dominant. Segregation for peridial color in the F_2 of the interspecific cross occurred in ratio of 35:29:26, respectively, for reddish brown, grayish brown, and gray. Tyler (118) and Vaheeduddin (122) concluded that there was tendency for brown color to be dominant in *S. sorghi*. Rodenhiser (92) stated that the intensity of color of the peridium varied somewhat, depending on the environment in which the plant was grown.

Vaheeduddin (122) found that dicaryophytes homozygous for brown peridium were stable, while those heterozygous for brown-gray were variable. Thus, differences in peridial color may be produced by the same dicaryophyte on different varieties and sometimes even on the same variety. The peridial color is determined by the genetic factors of the host, the genetic factors of the particular dicaryophyte, and the interaction of the two sets of factors under a given environment. The evidence indicates that variation in peridial color depends on a delicate balance of factors for color in the dicaryophyte.

The data obtained by Rodenhiser (92) indicated that the factors governing the general morphology of the smut sori, the color of the peridium, and the degree to which the host plant may be stunted

were inherited independently of each other. Tyler (118) also noted that the F_1 sori of *Sphacelotheca sorghi*, produced as a result of inbreeding and outbreeding of monosporidial lines, differed considerably in respect to hardness, size, shape of sorus, and color of peridium.

Nature of Germination. It is well known that environment at the time of spore germination has a profound effect on the type of germination (55, 58, 106). Equally important is the genetic make-up of the particular chlamydospore. Goldschmidt (38), in 1928, was the first to note that races of smut used in crossing influenced the nature of germination. He crossed monosporidial lines from different races of *Ustilago violacea* and obtained marked variation in degree of branching of the promycelium and also in the relative size of the promycelium. In fact, the promycelia of certain hybrid chlamydospores were much longer than those of either parent. Goldschmidt attributed this abnormal type of germination to the mixing of incompatible cytoplasm. Since then many types of abnormal germination have been reported, some of which have been shown to be inherited (20, 61, 72, 116). Vaheeduddin (121) found striking hybrid vigor in the promycelia from F_1 chlamydospores from a cross between *Sphacelotheca cruenta* and *Sorosporium reilianum*, and sporidia also were larger than those of either parent. The number of factors involved has not been determined. Recently, Laskaris (72) found that the promycelium from chlamydospores of crosses between certain monosporidial lines of *Sphacelotheca sorghi* were abnormally large.

Sporidial Production. Several workers (41, 110) have noted that the tendency for certain smuts to produce sporidia in culture is inherited. Popp and Hanna (83) made all possible crosses between sporidial and mycelial lines of *Ustilago levis* and concluded that the capacity for sporidial production in *U. levis* on nutrient media was definitely inherited and governed by a single factor. There was no linkage with sex or cultural characters.

Sometimes hyphal branches or peg-like structures develop on promycelia in place of sporidia. Rodenhiser (91) noted that certain crosses between monosporidial lines of *Sphacelotheca sorghi* and *S. cruenta* develop such structures. Vaheeduddin (121) encountered this type of segregation in a cross between *Sorosporium*

reilianum and *Sphacelotheca cruenta*. By inbreeding certain lines of *S. sorghi*, Laskaris (72) obtained chlamydospores that develop in most cases hyphal branches.

Christensen (20) also observed irregular development of sporidia in a *Ustilago zaeae* cross. In this case the promycelia were very inconsistent in the production of sporidia. Some gave rise only to sporidia, while some developed only peg-like hyphae; however, most of them gave rise to both types.

Kernkamp's inheritance study with *Ustilago zaeae* (61) proved that there was a clear-cut segregation on the promycelium of certain chlamydospores for production of sporidia or hyphal branches. Segregation on individual promycelia occurred on a 4:0, 3:1, 2:2, or 1:2:1 basis, with more than two factors being involved. He distinguished three types of growth: (1) a sporidial, (2) a mycelial, and (3) various intermediate types. The intermediate growth types could be shifted from sporidial to mycelial and back again, depending on environmental conditions, while the other two types of growth were more stable. The intermediate type of growth is another example of a character determined by a delicate balance of factors.

Lethals. Holton (51) and Popp and Hanna (83) experienced considerable difficulty in culturing single sporidia from F_1 chlamydospores resulting from crosses between *Ustilago avenae* and *U. levis*. The hybrid chlamydospores germinated normally but the sporidia did not grow on nutrient media, although some budded once or twice before they disintegrated.

Holton (51) crossed a monosporidial line of a buff smut with *Ustilago avenae* and *U. levis* and found that hybrid chlamydospores germinated normally; but none of the sporidia isolated from the crosses involving *U. avenae* grew in culture, whereas about 50 per cent of the sporidia isolated from a cross with *U. levis* grew perfectly well. Rodenhiser (92) also encountered sterility in interspecific crosses of sorghum smuts. But sporidia from interspecific and even intergeneric crosses sometimes grow perfectly well, so that failure of sporidia to grow can not be used as the sole measure of determining wide crosses (12, 121). Moreover, lethal sporidia have been observed as the results of close inbreeding. For example, in a cross between two haploid lines of *Ustilago zaeae*, in which nearly 100 per cent of the chlamydospores germinated and produced pro-

mycelia, only a small percentage developed sporidia capable of growth on artificial media. Most of the promycelia in this particular cross were pointed at the distal end, instead of blunt, and some developed hyphal branches instead of sporidia. Many of the sporidia failed to grow or else budded off only a few sporidia and then died. The hyphal outgrowths from the promycelium behaved in a somewhat similar manner (20).

Chilton (17) studied lysis in *Ustilago zeae* and showed that a high percentage of chlamydospores from certain crosses between haploid lines germinated abnormally and the promycelia usually disintegrated prior to the formation of sporidia. The promycelia that produced sporidia were atypical, the cells being large and irregular. A few sporidia grew well, while others budded a few times and failed to develop further. Similar results have been obtained by Laskaris (72) with certain inbred crosses of kernel smut of sorghum. Moreover, chlamydospores, promycelia, and sporidia in crosses involving lines containing lethal factors were significantly larger than chlamydospores from normal crosses (72).

Sex. Breeding tests indicate that factors for sex in some of the smut fungi are inherited in a definite manner. Kniep (65, 68) found that the segregation of sex factors in *Ustilago violacea* was in the ratio of 2:2 (2 plus and 2 minus lines). Similar sex ratios have been reported for *Ustilago hordei* (26), *U. avenae* (43, 48), *U. levis* (26, 43, 48) and *U. medians* (2). On the other hand, more complicated ratios have been reported for *U. zeae* (20, 41), namely, 4:0, 3:1, 2:2, 1:1:2, and 1:1:1:1. The 4:0 ratio may mean that all lines are solopathogenic or all incompatible on the basis of all possible pairings of sporidia from a single promycelium. Since the progeny from a single chlamydospore may all be intersterile, but some of them fertile with lines of other chlamydospores, it is sometimes necessary to cross monosporidial lines of one set with those from another chlamydospore in order to obtain a true index in segregation of factors for sex (20).

In 1923 Bauch (4) noted that there were three sexual groups in *Ustilago longissima*, and since that time more than two sex or compatibility groups have been reported for *U. zeae* (19), *Sphacelotheca sorghi* (92, 118), *Tilletia tritici* (36) and *T. levis* (36). Bauch (9) is of the opinion that there are only two sex groups in

U. zeae but that these are associated with seven sterility factors. However, numerous experiments have clearly shown that multiple factors for sex are involved in *U. zeae* and *S. sorghi* and, no matter what term is assigned to the phenomenon, its nature and its genetic explanation would not be simplified.

Cultural Characters. Extensive cultural studies of monosporidial lines of many species of smut indicate that collections of smuts frequently consist of heterozygous chlamydospores which give rise to numerous culturally distinct biotypes (10, 27, 57, 63, 103, 110, 120). Chlamydospores from most galls of *Ustilago zeae* are usually heterozygous for cultural character, but occasionally sporidial progenies from chlamydospores derived from the same gall are fairly uniform for certain cultural characters such as color and type of growth (20). However, numerous tests with many smuts indicate that monosporidial lines even from the same chlamydospores may differ strikingly in one or more of the following characters: color, topography, surface margin, consistency, rate of growth of colony, tendency to sector, response to temperature and response to H-ion concentration of the medium (2, 10, 20, 28, 41, 49, 91, 118).

Inheritance studies have been made of many of these characters. Such tests are made by crossing known lines and then analyzing their haploid progenies. Most of the cultural characters appear to be due to two or more factors. Complete sets of primary sporidia are isolated from individual chlamydospores and tested under conditions as nearly identical as possible. For instance, a cross between two distinct monosporidial lines, each of different color, may result in haploid lines with many new shades of colors because color is determined by more than one main factor and certainly by many modifying factors. The following segregation ratios from individual chlamydospores have been obtained for various cultural characters: 4:0, 3:1, 2:2, 1:1:2, and 1:1:1:1 (2, 20, 27, 41, 49, 91, 118). Within a species there may be many hundreds of different cultural types (20, 92, 108, 110).

Since there apparently are only two pairs of small chromosomes in most smuts, many factors must be carried in the same chromosome. Yet there is virtually no evidence that any of the cultural characters commonly studied are linked. Dickinson (28) concluded that color of colonies of *U. levis* was associated with two

additive linked factors, and Rodenhiser (92) obtained some indication for linkage between factors for reddish-brown peridia and parasitism.

Most workers (2, 20, 92, 110) agree that segregation of sex factors is independent of segregation of factors for culture and pathogenicity. Thus two monosporidial lines from the same chlamydospore may be alike in cultural characters, while in other cases they may be quite different. Consequently, Holton (51) was able to start with a single monosporidial line carrying a recessive factor for albinism, and develop albino races of oat smuts that were homozygous for chlamydospore color and that differed in pathogenicity from either parental race.

Pathogenicity. The dicaryophyte (with nuclei associated but not fused) is the parasitic stage of the smuts. The necessity of dicaryophytic hyphae for infection and development of chlamydospores was first noted by Zillig (132), and subsequently by many workers (2, 26, 36, 69, 109). However, fusion between sporidia and the initiation of the dicaryon is no assurance that infection and chlamydospores will result. In some cases the dicaryophyte may not extend beyond the fusion of sporidia or promycelial cells and the development of the infection hyphae (8, 14, 129). It is well known that certain smuts may enter the host but fail to develop chlamydospores; to this extent they lack either the factors for pathogenicity or their nuclei are not compatible enough to permit later fusion. Western (129) found that compatible lines of *Ustilago avenae* could enter and develop for a while in certain varieties of oats but were unable to develop chlamydospores. Moore (77) found that different combinations of monosporidial lines of *U. zeae* differed greatly in their ability to form chlamydospores on corn plants, although some combinations could produce large galls. In fact, one combination rarely formed chlamydospores and then only in an occasional gall.

Goldschmidt (38), in 1928, showed that the mating of particular monosporidial lines of *Ustilago violacea* influenced profoundly their parasitic action. His data indicated that association of two haploid nuclei of different parasitic abilities may have physiologically the same effect as if they had fused to give a diploid stage. The dicaryophytic hybrid produced from crossing monosporidial lines

from two different races was capable of attacking the hosts susceptible to both parental races.

In 1929 Hanna (41) and Stakman *et al.* (110) found striking parasitic differences in progenies from individual chlamydospores from field collections and also from galls resulting from crossing two monosporidial lines. Furthermore, there were marked differences in parasitism between solopathogenic lines derived from the same cross (19). The sporidial progeny from a chlamydospore of a solopathogenic line also may differ greatly in parasitism (20). It has been rather definitely shown that a monosporidial line of *Ustilago zeae* may be strongly pathogenic in combination with certain lines of opposite sex but only weakly so with other lines (110). Obviously, the pathogenicity of combinations is determined by the component of the factors present in two haploids.

Nicolaisen (81) obtained striking results in pathogenicity by inbreeding and outbreeding monosporidial lines of *Ustilago avenae*. Certain combinations of lines might cause heavy infection on one variety which was resistant or immune to other combinations of compatible lines from the same promycelium. Similar differences in pathogenicity were obtained by crossing monosporidial lines derived from different chlamydospores. His results, on the basis of extensive pairing of compatible lines, indicate that the factors for pathogenicity in the nucleus of one of two monosporidial lines making up the dicaryophyte may be dominant on one variety, recessive on another, and intermediate on others. Combinations were produced which differed in virulence from both of the original parents. Holton (51) obtained similar results with the oat smuts.

Allison (2) showed that segregation of factors for pathogenicity occurred in the promycelia of F_1 chlamydospores (cross between *Ustilago hordei* and *U. medians*). The F_2 dicaryophytes differed considerably in virulence on the five varieties of barley tested. Furthermore, the F_2 dicaryophytes did not have the same virulence on the varieties as the F_1 dicaryophyte or either of the parental combinations. It is rather significant that some of the new combinations, the F_2 dicaryophyte, were more virulent on certain varieties than the parental lines. As previously mentioned, there was considerable variation in head type of smut. One of the F_2 dicaryophytes smutted only the basal portion of the head, and in this respect it also differed from the other F_2 dicaryophyte.

Studies on the virulence of smuts have clearly shown that the inheritance of pathogenicity in smut is rather complex and that there is need for much more investigation in this field. It is clear, however, that pathogenicity for a specific variety is dependent on the association of nuclei with certain genetic factors. It also is apparent that the dicaryophyte has the potentialities of a hybrid, "plasma-hybrid," whose nuclei may fuse in the chlamydospore stage. Subsequent segregation makes possible new combinations which may differ greatly in virulence from either of the original parents. Such races have been experimentally produced.

Recently, Vaheeduddin (122) reported a new pathogenically distinct race of *Sphacelotheca sorghi* developed by crossing monosporidial lines derived from the promycelia of chlamydospores obtained from a single smutted kernel. Holton (51) compared the pathogenicity of pure lines, hybrids, and hybrid segregates of *Ustilago levis*, *U. avenae* and the so-called buff smut organism that originated from a mutation in *U. levis*. His data show clearly that a new pathogenic strain of *U. levis* that attacks the varieties Gothland and Monarch was produced by crossing a Gothland strain of *U. avenae* with a Monarch strain of *U. levis*. Also it is evident that a new pathogenic strain of the buff smut fungus that attacks both Gothland and Monarch was produced by crossing the Gothland strain of *U. avenae* with the Monarch buff smut strain which would not infect Gothland. As regards the bunt smut organisms, *Tilletia tritici* and *T. levis*, fertile hybrid chlamydospores have been produced by several investigators (10, 36, 42, 53). In this connection, Holton (53) found a cross between *Tilletia tritici* (race T-9) and *T. levis* (race L-8) to be pathogenic on the variety Oro which, heretofore, has been found to be susceptible only to *Tilletia levis* (race L-8). Thus there originated by hybridization a new race of *T. tritici* having some of the factors for pathogenicity of the *T. levis* (race L-8) parent.

Mutation

Frequency of Variations. Variation is a common occurrence in certain smut fungi, being especially common in *Ustilago zeae* and in *Sphacelotheca sorghi* (57, 92, 110, 118). In 1925 Bauch (5) reported a variant in *Ustilago longissima*. Since then variants have been reported in *Ustilago zeae* (110), *U. avenae* (49), *U. levis*

(49), *U. hordei* (2), *U. medians* (2), *Sorosporium reilianum* (38) and *Tolyposporium filiferum* (59).

The number of sectors that may appear in a single colony may vary from one to many. As a consequence, an indefinite number of variants may be produced. From a single monosporidial unisexual line of *Ustilago zeae*, Stakman *et al.* (110) isolated more than 150 distinct variants; and from another monosporidial line 70 different variants were obtained. Although the production of variants by smuts in culture may appear to be a rather common phenomenon, the frequency of change is not nearly so high as it seems, because there are billions of individuals in a single sporidial culture 4 cm. in diameter. Therefore the frequency may not be as great as in certain species of the higher plants.

It is not at all surprising that variation occurs so frequently in smuts growing in culture, because they are really growing under extremely artificial conditions. It has been repeatedly demonstrated that environment, particularly nutrition and temperature, plays a prominent part in inducing variation in fungi. This appears to be equally true for smuts (110, 118).

Types of Variants. Variants usually arise as wedge- or fan-shaped sectors in colonies growing on nutrient agar, and less commonly as "patches" on the surface of the colonies (110). The sectors and patch-variants may vary greatly in shape, size, color and other cultural characters. The variants in a parental colony may all appear alike or they may be quite different as to color, rate and type of growth, *etc.* A variant may be restricted to a small portion of the colony or it may occupy so large an area that it is almost impossible to differentiate by size of colony between the variant and parent. Sometimes the variant may even occupy the greater portion of the colony (20, 92, 110).

Variants, when compared on nutrient agar, differ from each other and from their parents in the following characters: type and rate of growth, color, topography, consistency of colonies, and tendency to sector. Some also differ in parasitism when mated to certain testers of opposite sex. The extent of variation in cultural type is sometimes very great. For instance, color may vary from white to almost black, and the type of growth from pure sporidial to mycelial. Changes may occur in factors for chlamydospore color

and morphology of sporidia (5, 20, 110). However, variations are not always so pronounced; in fact, Stakman (108) has recently pointed out that in *Ustilago zeae* numerous small, almost imperceptible variations occur commonly. Many variants of this type are perhaps overlooked, and yet they may play a very important role in the evolution of the smuts.

Tendency to Vary. There is a marked difference in the tendency of different species of smuts to sector. *Ustilago zeae* and *Sphacelotheca sorghi* are especially prone to sector in culture, while *U. longissima* and *Tilletia tritici* apparently are more stable in this respect. Monosporidial lines within a species may differ greatly in their stability, i.e., some mutate frequently, others seldom. Stakman (107, 108) has made a special study of mutability of monosporidial lines in *Ustilago zeae*. Crosses were made between constant by constant and mutable by mutable, and their progeny studied. He proved that in certain lines the factors for tendency to mutate were definitely inherited and that segregation for variability occurred in definite ratios (107).

Stability of Variants. Some variants are stable and others are not. On the other hand, some variants are as stable as their parents, while still others are unstable and may continue to give rise to more variants (57, 92, 110). As a consequence, there may occur a series of successive variants, one arising from the other. Certain haploid lines may continue to give rise to variants for many years. Some lines are so unstable that it is virtually impossible to carry them in culture, at least one can not be certain that the original line is present, while some lines have been kept pure for many years. Thus Stakman *et al.* (112) found 14 variants of *Ustilago zeae* that maintained their characteristic features on artificial media for nearly five years. The pathogenicity of some of these variants also remained unchanged.

Breeding tests with variants, crossing them with certain haploid lines and studying their F_1 progeny, have definitely proved that variants arising in culture are the result of genotypic changes within the haploid nucleus (20, 108). Variants that arise in lines that belong to heterothallic species are perhaps not nearly so important as those of homothallic smuts, since in heterothallic lines there are more opportunities for variation as a result of natural crossing.

To some it may seem difficult to reconcile the complex inheritance and great variability of smut fungi with the small number of chromosomes. It is of course possible, although evidence for it is lacking, that the genetic behavior of the smuts may not in all respects be of the same pattern as that in higher plants and animals. However, it must be remembered that the evidence for the genetic behavior of the smuts has been obtained principally from the haploid generation, whereas in higher plants and animals the evidence has come primarily from the diploid generation. Moreover, in the smuts, it has been possible to study them under more closely controlled conditions than is generally possible with higher forms.

A general review of the work dealing with physiologic specialization and genetics of the smut fungi impresses one with the complexity of the smut problems. It is rather significant that the smuts were among the first fungi to be investigated, and yet today, after at least seventy-five years of intensive research, their parasitisms, their variability and their genetic behavior are still very imperfectly known. Recent investigations on the genetics of smuts, while adding much to our fundamental knowledge of the problem, have emphasized its great complexity and the need of further investigations.

SUMMARY

The term physiologic race has been used to designate groups within species and varieties that differ in one or more of the following characters: pathogenicity; cultural characters on artificial media; physiologic and ecologic characters; biochemical effects; and morphology. In the Ustilaginaceae it is defined on a convenience basis and used to designate a collection of chlamydo-spores that behaves more or less consistently in parasitism on certain differential varieties of host plants. Obviously the races that differ in pathogenicity rather than in other qualities are of greatest economic importance and offer the greatest difficulty in developing smut resistant varieties of cereals.

Many varieties of cereals believed to be smut resistant have been found, when grown commercially, to be completely susceptible to one or more races of the smut fungi. There is reason to believe that in the future these experiences will be reduced to a minimum. More information is available concerning the number, prevalence, distribution, and virulence of races of the smut fungi, and a number of

varieties and selections of cereals are now known to have factors for resistance to many of the races that are rather widely distributed. Thus, the plant breeder may more intelligently select parental material to be used in crosses and can recognize the limits within which his efforts are likely to be successful. Progress has already been made along this line in the development of the bunt resistant variety of wheat, Oro \times Turkey-Florence and also in the oat smut resistant progeny from a Markton \times Rainbow cross.

Apparent changes in pathogenicity of chlamydospores of a single smut collection have been attributed to (1) adaptation of the fungus or (2) the "screening" effect from a collection of chlamydospores comprising more than one race. The latter is undoubtedly more common. Thus screening may effect separation of mechanical mixtures of chlamydospores of different races and also progenies from individual chlamydospores heterozygous for pathogenicity.

Certain smut fungi, particularly those that produce true sporidia, are especially suitable for studies of heritable variations. Complete sets of unisexual sporidia can be isolated from individual promycelia and propagated indefinitely on nutrient media. This makes possible genetic study of numerous haploid lines under controlled conditions. Most species of smuts are, or have the potentiality of becoming, heterothallic. Consequently, genetic studies also can be made of the parasitic behavior of dicaryophytes derived from nuclear associations of two haploid lines.

The type of germination depends upon the genetic constitution of chlamydospores and upon the environmental conditions at time of germination. Chlamydospores of different species of smut and even of different collections of the same species may germinate quite differently with respect to type of promycelium, number of septa, and sporidia produced. In many species the production of sporidia on the promycelium is often very irregular.

Most species of smut possess two pairs of chromosomes, but relatively little is known regarding their behavior. Reduction occurs usually in the first or second division of the fusion nucleus, but there is some evidence that it also may occur in later nuclear divisions in the promycelium. Occasionally, no reduction takes place and in such cases the sporidia are uninucleate and diploid and possess parasitic capabilities similar to dicaryonts. Reduction may not necessarily be complete in one mitotic division, as some factors may

reduce at the first division and others at the second. Crossing over apparently occurs.

Inheritance studies have shown the following characters to be inherited: size, color, and wall markings of chlamydospores; nature of chlamydospore germination with respect to degree of branching and production of sporidia, disintegration of promycelia (lysis), size of sporidia and promycelia, tendency for sporidia to bud in culture, production of sporidia that are lethal; color, topography, margin, consistency, and rate of growth of colonies in culture, and tendency of lines to sector on nutrient media; sexual compatibility and pathogenicity; and general morphology and consistency of smut sori, color of peridium of sori, and degree to which the infected host plant may be stunted.

Segregation of factors for many of the characters occurs on individual promycelia in the following ratios: 4:0, 3:1, 2:2, 1:2:1, and 1:1:1:1. Most of the characters that have been studied are apparently inherited independently of sex and of one another. Some characters such as color of chlamydospore and wall markings are determined by single factor differences, while other characters, *e.g.*, color of colonies, and pathogenicity of lines, are governed by multiple factors. In some species (*Ustilago hordei* and *U. avenae*) sexual compatibility is determined by a pair of factors; in others (*Ustilago zeae* and *Sphacelotheca sorghi*) multiple factors are involved.

New parasitic races of smut may arise through hybridization. In heterothallic species of smut, fusion of haploid lines of opposite sex is essential for normal infection and production of chlamydospores; consequently, hybridization between different biotypes of the same species is a rather common phenomenon. Interspecific hybridization between haploid lines is not uncommon. For instance, numerous crosses have been made between *Ustilago hordei* and *U. medians*, *U. levis* and *U. avenae*, *Tilletia levis* and *T. tritici*, and *Sphacelotheca sorghi* and *S. cruenta*. In addition, parasitic progenies from two intergeneric crosses have been reported; *i.e.*, *Sorosporium reilianum* \times *Sphacelotheca sorghi*, and *S. reilianum* \times *S. cruenta*.

New biotypes also may arise by mutation. Variants occur frequently in haploid lines of certain smuts, but not in others. These arise usually as wedge- or fan-shaped sectors, or patches, in colonies growing on nutrient agar. Variants may differ from each other and

from their parents in the following characters: type and rate of growth, color, topography, consistency of colonies, tendency to sector, morphology of sporidia, sexual compatibility, and parasitism. Hybridization studies indicate that the new characters are heritable. Obviously mutation makes possible new combinations of biotypes and hence leads to greater variation in the species.

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THE RELATION OF VIRUSES TO PLANT TISSUES

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INTRODUCTION

The subject of the relations of viruses to plant tissues has been approached from several different angles, beginning with the early work of Allard (1915) who showed that the virus of tobacco mosaic invades nearly all of the plant parts.

More recent work has provided much additional information regarding the invasion of plants by the virus of tobacco mosaic and by numerous other viruses and has extended knowledge of the subject into the field of the relation of viruses to specific tissues of the plants involved. This information is scattered through a large volume of literature and much of it is incidental to the consideration of other phases of the diseases resulting from virus activity.

It is the purpose of this paper to assemble as much as possible of the evidence bearing on the relation of plant viruses to specific tissues and to point out some of the deductions that may seem justified by the information that has been made available up to the present time.

TISSUES INVADED BY VIRUSES

It is generally considered that plant viruses, with a few notable exceptions, are systemic and that when introduced into a susceptible plant they usually invade all of the plant parts. However, even with so-called systemic viruses there is evidence that the various types of tissues differ in the amounts of resistance offered to invasion. This resistance varies, depending on the plant and on the virus involved. Since, so far as known, viruses do not normally invade non-living tissues, a discussion of the invasion of plants by viruses may be limited to a consideration of the three major types of living tissues, namely, meristematic, parenchymatous, and conductive tissues.

Meristem. The determination of the presence or absence of virus in meristematic tissue offers certain difficulties that, although probably not insurmountable, have not yet been satisfactorily overcome. Maturation is so rapid at the growing points that only small quantities of meristem are available for direct determination of virus content. Isolation of relatively small groups of meristematic cells with no contamination from adjacent tissues requires patience and skill. However, it would seem that a direct determination of the presence or absence of virus is a possibility in the meristem of root tips and in certain types of callous formations.

There is evidence tending to indicate that after introduction into any part of the plant, viruses usually move rapidly to the growing points where symptoms appear first on the new growth. Other evidence indicates that sometimes young succulent parts are more susceptible to infection than older more mature parts. These considerations have led some investigators to assume that rapid multiplication of viruses is associated with relatively large quantities of meristem. Caldwell (1931) states that it is generally conceded that presence of meristematic tissues is necessary for active multiplication of the virus or tobacco mosaic and suggests that slight tendency to faster upward movement found with this virus may be associated with the increased rate of multiplication of the causal agent in meristematic tissue. However, it is well known that this virus is able to multiply in mature leaves and stems.

Matz (1934) found that the juice from the rolled inner white and brittle portions of leaf bases in the lower regions of the pseudostem of sugarcane plants affected by sugarcane mosaic, apparently had a lower concentration of virus than juice from mature green leaves, midribs, leaf sheaths, and true stems. Also (Matz 1935) the white, brittle portions of the inner leaf bases immediately above the apex of the stem gave evidence of being less receptive to infection in artificial inoculation tests than surrounding tissues. Juice from green parts of diseased plants mixed with juice from immature parts of the pseudostem of healthy plants was less infectious than mixtures of juices from the green parts of diseased and healthy plants. This evidence indicates that meristematic tissue of sugarcane is unfavorable to the virus of mosaic.

Grainger (1934) emphasized the lack of direct evidence that would indicate that viruses are able to enter meristematic tissue.

More recently, however, Lackey (1938) reported finding relatively high concentrations of the virus of curly-top in the root tips of beets and beans beyond the region of differentiating phloem. If this virus occurs in meristem of root tips in the relative concentrations suggested, it is evident that the meristem of the root tips of beet and bean plants has a considerable degree of permeability as well as great resistance to injury. The close association of the virus with the phloem in the more mature parts of the plant indicates that the virus is inactivated in most of the tissues derived from infected meristem.

Sheffield (1933) found that in certain Solanaceous plants affected by aucuba mosaic, the meristematic tissue of diseased plants appeared to be like that of healthy plants. Incipient inclusion bodies were not found until plastid development was well advanced, but the virus apparently entered some of the cells early enough to inhibit development or cause destruction of plastid primordia, thus giving rise to the chlorotic leaf areas. In the green areas the plastids were normal, indicating that the virus entered the cells at a later stage of development. Under such conditions it must be assumed that the more actively dividing meristematic cells were free of virus.

Valleau (1935) states that the meristematic tissue of the growing point of tobacco plants appears to be nearly immune to the virus of tobacco mosaic, and suggests also that failure of this virus to enter the seeds may be the result of inability of the virus to invade meristematic tissue.

Apparently there is no evidence of a cytological nature that indicates the presence of any virus in cells of the meristematic regions of affected plants. The continued normal functioning of the meristem of the growing points and cambium regions of most virus-affected plants, indicates that if viruses are present in the meristem they rarely cause appreciable direct injury to this type of tissue.

The more rapid movement of viruses from points of introduction to the growing points of plants that is found frequently may be associated with the transport of elaborated foods to these regions. This possibility is discussed further in another section of this paper.

*Parenchyma.*¹ Various types of parenchymatous tissues are un-

¹ In this paper the epidermis, although not usually classified as parenchyma, is treated as if it were a type of parenchymatous tissue. This seems justified in the interest of brevity and simplicity, since the epidermis partakes of most of the characteristics of parenchyma and since its relation to viruses is similar to that of true parenchyma.

doubtedly extensively invaded by certain plant viruses. The rubbing of leaves of tobacco plants with a cloth saturated with juice from plants affected with common mosaic, results in infection. This method of inoculation introduces the virus into trichomes and other epidermal cells. The virus passes from the injured cells into cells of the palisade and mesophyll and finally enters the vascular elements through which it is transported rapidly to other parts of the plant. It seems certain that the virus multiplies in parenchymatous tissue and it seems reasonable to expect that all viruses that are capable of producing infection through injured epidermal cells are able to move and multiply in the ground tissue of the plant. It may be suspected further that all viruses that cause mottling or local lesions in leaves are able to invade parenchyma, even though they may not be readily transmissible by mechanical inoculation.

However, there are diseases, leaf-curl of raspberry, peach-yellows, sugar beet curly-top, aster-yellows, and others, caused by virus, that do not produce local lesions or mottling and with which infection through injured epidermal cells apparently does not occur. Where these viruses remain active in plant extracts, the rubbing method of inoculation mentioned in connection with tobacco mosaic undoubtedly serves to introduce them in an active state into epidermal cells. The obvious conclusion is that where such viruses remain active in expressed plant juice but fail to produce infection when introduced into parenchyma cells, they are probably unable to multiply in parenchyma tissue or to migrate through parenchyma from the cells into which they are introduced.

Other evidence supports the view that where infection by mechanical means is difficult, often little or no virus occurs in the parenchyma. *Eutettix tenellus* (Baker), the vector of the virus of curly-top of sugar beet, rarely obtained virus when its feeding was restricted to parenchyma of diseased plants (Bennett, 1934) or when its feeding was restricted to extracts of juice from parenchyma of diseased plants (Bennett and Esau, 1936). Also, the virus failed to pass through the woody cylinder of tobacco stems in periods some of which were longer than one year. Although this evidence does not prove complete absence of the curly-top virus from all parenchyma, it indicates that, at most, not more than relatively low concentrations of virus occur in the types of parenchyma tested. It seems reasonable to suspect that a number of viruses of this general type may have similar tissue relationships.

Vascular tissue. The vascular bundles of plants serve to rapidly transport viruses to various parts of the plant, and in some instances they appear to be the sole channels for virus transport. Due to differences in anatomical structure and physiological functioning of the xylem and phloem of vascular bundles it would be expected that these two parts of the conductive strands would bear very different relations to viruses.

Certain investigators have suggested that the xylem may be the path of dispersion of viruses through the plant, but little direct evidence supporting this view has been presented. Johnson and Mulvania (1924) attempted to force virus of tobacco mosaic from the xylem of tomato plants through the hydathodes of the leaf by placing the root system under a hydrostatic pressure of 200 pounds per square inch. Liquids obtained from the hydathodes by this method proved to be infectious but contained less virus than extracted plant juice. It seems probable, as Johnson and Mulvania point out, that the liquid from the hydathodes may have been contaminated by content of living cells injured by the high pressures employed.

Later work by Caldwell (1931) has shown that water naturally guttated from tomato leaves affected by aucuba mosaic contained no virus, whereas liquid guttated under pressure contained virus. Grainger (1933) obtained similar results using the virus of tobacco mosaic in tomato.

Other evidence indicates strongly that viruses are not normally found in the tracheae of the xylem, but there is some indication that they may occur in the xylem parenchyma. Numerous attempts have been made to infect plants by filling the tracheae with liquids in which viruses were suspended. However, infection has not resulted from this method of introducing viruses into plants when the plants were not injured after the viruses were introduced. Caldwell (1930, 1931) induced liquids containing the virus of aucuba mosaic to enter tomato plants through the cut ends of petioles and pass to various parts of the plant through the xylem. No symptoms of disease appeared when the plants were not injured further. However, infection was produced readily when the xylem was crushed and the tracheal content allowed to escape into adjacent tissues. When this virus was inoculated into plants by the usual method of rubbing, it did not pass a part of the stem that had been killed

by steaming. When placed in the xylem, however, the virus passed the steamed areas and caused symptoms on the other side of the steamed areas when released from the tracheae through injuries. Similar results were obtained with steamed stems of tobacco using the virus of tobacco mosaic.

This evidence shows that these viruses were unable to pass out of unbroken tracheae and enter adjacent cells. Conversely, it does not seem probable that they would be able to pass from living cells into tracheae. This indicates that viruses do not occur normally in that part of the xylem chiefly concerned with the movement of water and mineral elements. However, Matsumoto and Somazawa (1933) presented evidence indicating that the virus of common mosaic of tobacco occurs in the woody cylinder of tobacco plants. In this case the virus was present probably in the wood parenchyma and in the medullary rays. Bennett (in press) found that the virus of tobacco mosaic is able to pass either longitudinally or radially through the woody cylinder of stems of Turkish tobacco. This was true also of the virus of cucumber mosaic in *Nicotiana glauca*. Perhaps viruses that occur generally distributed in parenchyma would be expected to invade parenchyma of the xylem regions.

Evidence of a close relationship between viruses and the phloem portion of the vascular bundle is very strong. The majority of insects that are vectors of plant viruses seek out and feed on the phloem. This is strikingly evident in the case of vectors that transmit viruses not easily transmissible by mechanical means. Certain viruses, such as the virus of spotted-wilt transmitted by *Frankliniella insularis* (Bald and Samuel, 1931) and the virus of pineapple yellow-spot transmitted by *Thrips tabaci* (Linford, 1932), are transmitted by insects that presumably feed on parenchyma, but these viruses are also transmissible by mechanical means, and introduction of virus into parenchyma is probably sufficient for infection.

Most insects that feed on the phloem are admirably equipped by nature not only to remove large quantities of material from the phloem but also to introduce appreciable quantities of their own secretions into the phloem as well as into cells of surrounding tissues. The marked effectiveness of phloem-feeding insects as vectors and the complete dependence of certain viruses on this type of vector for dissemination point strongly to an intimate relationship between the phloem and the viruses that are transmitted.

Holmes (1932) showed by the starch-pattern method of following virus movement that the virus of tobacco mosaic bears a very decided relation to the veins of the leaf in some of the earlier stages of its invasion of the plant. Samuel (1934) found a similar condition in tomato plants affected with tobacco mosaic. The rate of movement following contact of the virus with the veins and the subsequent path of movement clearly indicate that the faster rates of movement occur in the veins. In view of the evidence indicating absence of virus in the tracheal elements of the xylem, it seems evident that the rapid movements of these viruses through leaves and stems occur in the phloem.

Ringing experiments show (Bennett, 1927) that the leaf-curl virus of raspberry is unable to move through the woody cylinder of raspberry canes. Somewhat similar experiments indicate (Bennett, 1934) that the virus of curly-top is unable to move longitudinally or laterally through the woody cylinder of *Nicotiana glauca* or *N. tabacum* but that it passes readily from internal to external phloem, or *vice versa*, through the union of the two types of phloem in the leaf traces. As already pointed out, the curly-top virus occurs in very low concentrations, if at all, in the cells of the parenchyma of the petiole, pith of the crown, and flowering stalk of beet. Exudate produced naturally on diseased petioles and probably derived originally from the phloem and exudate derived from the severed ends of vascular bundles of diseased beet roots has a very high virus content.

With most viruses the evidence points to the phloem as the tissue through which rapid invasion takes place and in some plants the tissue in which virus occurs in greatest concentration. The phloem is apparently well adapted to the rapid distribution of virus to all parts of the plant when conditions are favorable for movement.

CLASSIFICATION OF RELATIONS

Approaching the subject of tissue relations of viruses from a somewhat different viewpoint and considering only phloem and parenchyma, viruses seem to exhibit three main relationships to plant tissues. These relations may be classified as follows: (1) a relation in which virus is more or less restricted to parenchyma, (2) a relation in which virus is more or less restricted to the phloem, and (3) a relation in which virus occurs extensively distributed in both phloem and parenchyma.

Restriction to parenchyma. A virus restricted to parenchyma in all of its host plants would be greatly handicapped. Movement through the parenchyma is known to be relatively slow, and the time required to invade all of the parts of a plant by travel through the parenchyma probably would be quite long. Thus, the amount of inoculum would be more limited, and spread from plant to plant would occur less often than if the virus were able to invade the plant through the phloem. If a virus of this type were evolved it would require special conditions to enable it to survive, especially if not seed-transmitted and if its host plants were annuals.

The virus of tobacco necrosis, as described by Smith and Bald (1935) and by Smith (1937), shows some extremely interesting relations to infected plants and may prove to be closely limited to parenchyma. This virus is able to persist for long periods in certain greenhouse soils and is resistant to the ordinary agents in the soil. It attacks tobacco and a number of other plants but is usually restricted to the roots, although in young plants it may move into the stems and cause necrosis of stem and leaf tissues, sometimes resulting in death of the plant. When inoculated into leaves it causes necrotic lesions in a wide range of plants but produces systemic infection only in French bean (*Phaseolus vulgaris*). Since natural infection usually takes place from the soil and since the virus shows a high degree of restriction to roots, it seems probable that it is largely confined to parenchyma and unable to pass into the phloem and move rapidly into the tops of affected plants.

There is evidence that there are viruses capable of extensive tissue invasion in some of their host plants but restricted to parenchyma in other plants. Holmes (1929) showed that the virus of tobacco mosaic was usually localized in the inoculated leaf of *Nicotiana glutinosa*, and Caldwell (1932) found that the virus of aucuba mosaic produced local lesions in this species but was unable to produce systemic infection. Several other cases are known in which viruses produce local lesions on certain host plants but fail to invade the plant systemically.

There are viruses also that are able apparently to produce systemic infection under certain conditions but restricted to parenchyma under other conditions. Smith (1932) found when the virus of spotted-wilt of tomato was inoculated into petunia leaves by rubbing or by means of the insect vector, *Thrips tabaci*, local lesions resulted,

but usually systemic infection did not occur. On the other hand, when the virus was introduced into the stems by needle punctures systemic infection resulted but no local lesions were produced. This seems to constitute a case in which the virus is capable of remaining active in either phloem or parenchyma but passes from one type of tissue to the other with difficulty. Rubbing the leaves with a virus suspension or inoculating them using thrips would ordinarily introduce the virus into the more superficial parenchyma cells. In these cells it apparently multiplied and caused necrotic areas, but since systemic infection was not produced it probably did not enter the phloem. Introduction of the virus into the stem through needle punctures might, in some instances at least, place the virus in the phloem. From the point of introduction it would be carried to other parts of the plant and become systemic. From such systemic infections, however, local necrotic spots did not develop, which suggests that although the virus was able to invade the phloem network of the plant it was not able, at least in the leaves mature at the time of inoculation, to pass out of the phloem in quantities sufficient to produce local lesions.

Also in recent work it has been found that a strain of cucumber mosaic virus when introduced into mature leaves of sugar beet by rubbing produces only local lesions. The local lesion phase of the disease can be perpetuated indefinitely. When the virus is introduced into the plants by aphids, however, a systemic infection frequently results that causes a severe type of mottling but produces no local lesions of the type resulting from inoculation by the rubbing method.

The chance of survival of a virus limited to parenchyma in all of its host plants would seem to be greater in perennial plants, especially in vegetatively propagated perennials. One virus which apparently has this limitation occurs in peach. Hutchins (1939) found that when whole root sections from peach trees having phony peach were grafted onto roots of healthy trees the disease was transmitted in all cases where union took place; but, when bark from diseased roots was grafted onto healthy roots no transmission occurred. This indicates that the virus of phony peach is restricted to the woody cylinder. Since it is reasonable to conclude that the virus is closely confined to living tissues, it seems probable that it moves and multiplies in the wood parenchyma, or in the medullary rays or both.

The tissue relationships of this virus are all the more interesting because of the fact that it is apparently not present in the tops of affected plants (Hutchins, 1929). Why the virus should occur in the woody cylinder of the roots and not in the woody cylinder of the limbs is an interesting question. Kunkel (1935) suggested that the virus may be inactivated in the parts above ground during periods of high summer temperatures. Hutchins and Rue (1939) presented evidence indicating that the virus is destroyed by subjecting dormant infected trees to a temperature of 48° C. for a period of 40 minutes. The effect of lower temperatures over longer periods has not been determined. It may be pointed out, however, that since the virus is presumably limited to parenchyma, spread probably is slow, and several months might be required for it to move from the roots to the tops of an average size tree, even under conditions favorable for maximum rates of movement. Therefore, summer temperatures high enough to cause inactivation of the virus in the above ground parts would be expected to cause restriction of the virus to the roots and lower parts of the trunk.

Restriction to phloem. There are probably several viruses that are more or less closely limited to the phloem in their increase and movement in the plant. Of those that probably have this limitation, the virus of raspberry leaf-curl and the virus of sugar beet curly-top have been studied most extensively in this connection. Studies (Bennett, 1927, 1934) have shown that these viruses may be confined to certain parts of an infected plant by destroying the phloem connections between the inoculated portion and other parts of the plant at the time of inoculation.

The virus of curly-top passes readily through either the internal or the external phloem of the stem of both *Nicotiana tabacum* and *N. glauca*, but it is unable apparently to pass from one type of phloem to the other through the medullary rays or other tissues normally found in the woody cylinder of the internodes of the stem, although it makes this transition without any measurable delay by means of the union of the external and internal phloem in the leaf traces. In both beet and tobacco the virus of curly-top occurs in relatively high concentrations in the phloem and is absent from or present in only low concentrations in the parenchyma adjacent to vascular bundles of the petiole, crown, and flower stalk. In beets affected by curly-top, Esau (1933) observed necrosis only in the

primary and secondary phloem and pericycle. She interpreted changes occurring outside the phloem as secondary responses to necrotic conditions in the phloem and concluded (1933, 1935, 1935a), on the basis of extensive anatomical evidence, that the virus is active mainly in the phloem.

It seems probable that there are other diseases besides leaf-curl of raspberry and curly-top of sugar beet that are caused by viruses closely limited to the phloem. This conclusion is reached on the basis of the characteristics that diseases caused by phloem-limited viruses would be expected to manifest.

In general, symptoms should be those characteristic of diseases arising from disturbances in the phloem. Typically they might include phloem necrosis, vein distortion, leaf rolling, curling and crinkling due to growth disturbances in the veins, and yellowing and dwarfing of parts and of entire plants, but probably no mottling of the mosaic type would be expected. Viruses of this type would not be seed-borne, for, since they do not occur in tissues outside the phloem, they would not be able to enter the gametes or to pass through the meristematic or parenchymatous bridge separating the mother plant from the young sporophyte. It is significant in this connection that no virus which does not produce local lesions or mottling has been shown to be transmitted through seeds.

Transmission by placing virus in the superficial cells of the leaf, as in the rubbing method of inoculation, would not result in infection, and inoculation by means of needle punctures would rarely produce infection since it seems to be difficult to introduce virus directly into the phloem. It is recognized, however, that certain viruses that probably are not limited to the phloem also fail to produce infection when introduced into epidermal cells, perhaps because of rapid inactivation when removed from the living cell or inability to reproduce and move in the presence of products of the injured cells into which they are introduced. For this reason, failure of mechanical inoculation to produce infection, although characteristic of phloem-limited viruses, can not be accepted as conclusive evidence that a specific virus is limited to the phloem.

Viruses that are limited to the phloem should be almost exclusively insect transmitted, and vectors should be relatively few in number and exhibit a greater degree of specificity in virus transmission than is found in vectors of viruses not limited to the phloem.

It seems probable that only insects that habitually feed on the phloem and that permit virus to pass through their bodies and to be injected into the plant through the medium of their saliva, would qualify as effective vectors.

With these points in mind, it seems probable that such diseases as peach-yellows, little-peach, peach-rosette, potato leaf-roll, yellow-dwarf of potato, aster-yellows, cranberry false-blossom, peanut-rosette, and spike-disease of sandal are caused by viruses that may be more or less closely limited to the phloem. Indirect evidence, recently published, supports this view in respect to peach-yellows, little-peach, peach-rosette, and potato leaf-roll.

Kunkel (1938), in the transmission of virus diseases of peach by budding, found that the contact period between bud and twig required for mosaic transmission was usually 2 to 3 days, whereas the contact period required for the transmission of yellows, little-peach, and rosette was 8 to 14 days. Since the virus of mosaic would be expected to occur in the parenchyma, it would pass out of the infected buds into healthy tissue as soon as parenchymatous bridges were available, whereas the viruses of the other three diseases, if restricted to the phloem, would pass from infected buds into healthy tissue only after phloem bridges were available, which would be sometime after parenchymatous unions had been formed.

In the transmission of potato viruses by vectors, Dykstra and Whitaker (1938) found that four species of aphids, *Myzus persicae*, *M. solani*, *M. circumflexus*, and *Macrosiphum (Illinoia) solanifolii*, under certain conditions are effective vectors of certain mosaic viruses of potato. The first three were also effective vectors of the potato leaf-roll virus but the fourth generally failed to transmit the leaf-roll virus though occasionally a fairly high percentage of infection was obtained. The first three species named above were found to feed on the phloem but the fourth species fed on the phloem in less than 50 per cent of the cases noted. A feeding relation of this latter type would be expected to result in reduced efficiency in the transmission of a phloem-limited virus.

The yellow-dwarf virus of potato, transmitted by the leafhopper *Aceratagallia sanguinolenta*, may furnish an example of a virus closely limited to phloem only in certain hosts. Black (1938) showed that on potato and crimson clover the virus is transmissible with extreme difficulty, if at all, by the ordinary methods of inocu-

lation that serve only to introduce viruses into epidermal cells; whereas these methods were very effective in transmission of the virus to a certain variety of *Nicotiana rustica*.

It was concluded that probably the main requirement for infection of potato and crimson clover is introduction of the virus into the phloem, and that for infection of *N. rustica* introduction of the virus into the phloem is not necessary.

Symptoms of the disease on potato and crimson clover are typical of those caused by phloem-limited viruses and consist of yellowing and dwarfing in potato and of vein clearing, yellowing, and dwarfing in crimson clover. Symptoms on *N. rustica* are more typical of those caused by parenchyma invasions and consist of yellow spotting and necrotic lesions. Systemic infection indicates the virus also occurs in the phloem. The relatively high concentration of virus found in juice from infected plants of *N. rustica* as compared with concentrations found in juice from potato and crimson clover is further indication that the virus is more extensively distributed through living tissues in *N. rustica* than in potato and crimson clover.

Occurrence in both parenchyma and phloem. Perhaps common tobacco mosaic is the best known example of a virus disease in which the causal agent occurs in both parenchyma and phloem, but there are numerous other viruses that have a similar tissue relationship. The virus of tobacco mosaic produces infection through trichomes or other epidermal cells and passes from the epidermis through other types of parenchymatous tissue before it reaches the phloem in which it evidently travels at its most rapid rate.

Holmes (1932) has shown that the rate of initial spread following introduction into epidermal cells is slow and roughly equal in all directions until the larger veins are reached. After the virus enters a vein it moves away from the original lesion very rapidly. A limited period of delay in entering small veins and a close association with the veins in the earlier stages of systemic invasion suggest the presence of cells around the phloem that are permeable to the virus with a certain amount of difficulty. The permeability of these cells apparently varies somewhat at different points, since Holmes has shown that as the virus passes away from the place of entrance it is at first closely restricted to the veins for the most part but escapes at intervals to widen considerably the path of

invasion in certain parts of the leaf. Samuel (1934) found a similar unequal spread of virus from the veins into the neighboring parenchyma of tomato leaves.

Probably all of the viruses that cause mosaic mottling first extensively invade plants through the phloem and later spread into other tissues where they continue to increase and often reach relatively high concentrations.

Viruses of this type might be expected to produce symptoms that would be evident in either parenchyma or phloem or both. However, the most evident symptoms in most instances seem to be those resulting from disturbances in the parenchyma and consist as a rule of local lesions of various types, and of mottling. Phloem necrosis and other phloem disturbances occur more rarely.

The inconspicuous character or absence of symptoms in the phloem of plants infected by viruses that cause mottling or local lesions suggests that some of these viruses may occur only in low concentrations in the phloem. Perhaps a virus that is equipped to multiply rapidly in an acid medium such as parenchyma would not multiply equally well in an alkaline medium such as the phloem. If the phloem content were decidedly unfavorable, movement through the phloem might occur only under special conditions. This may account for some of the cases of delayed systemic infection and partial invasion of plants by certain viruses.

Storey (1938) showed that *Cicadulina mbila*, the vector of the virus of streak-disease of maize, normally feeds on the phloem and must feed on the phloem in order to transmit. When it was allowed to feed on chlorotic areas of infected maize leaves it picked up virus but when its feeding was restricted to the normal green areas of the leaf it was unable to acquire virus from such areas. This was true regardless of whether feeding was confined to parenchyma or permitted to extend into the phloem. It seems quite improbable that the virus could be so localized that its occurrence in the phloem would be restricted to areas surrounded by infected parenchyma cells. It seems probable, therefore, that the insect acquires virus from infected parenchyma cells and that it cannot pick up enough virus from the phloem to enable it to become a vector, although the virus undoubtedly occurs in the phloem and moves in this tissue very rapidly. This evidence suggests relatively high concentrations of virus in the parenchyma of chlorotic spots, low concentrations or

absence of virus in normal green parenchyma, and very low concentrations of virus in the phloem.

Further information regarding the relative concentration of virus in phloem and parenchyma of plants affected by other viruses would be of interest. In certain plants this information could be obtained by comparing concentrations of virus in expressed sap with those in phloem exudate.

Viruses that occur in both phloem and parenchyma should be readily inoculable by mechanical means except with those that are easily inactivated by products of injured cells or by other factors encountered in the transfer of viruses from one plant to another.

Insects that feed chiefly on the phloem and that are able to pass viruses through their bodies and reintroduce them into the plant through the medium of their saliva would be effective in transmission of viruses that occur in both phloem and parenchyma. However, passage through the insect may not be so important as with phloem-limited viruses.

Doolittle and Walker (1928) showed that *Aphis gossypii* loses the virus of cucumber mosaic in the first or second short-interval transfer on healthy plants, and Bennett (1932) found a similar condition with *Amphorophora rubi* in its relation to the virus of mosaic of raspberry. Although it is possible that these results are due to the inability of the viruses involved to remain active in association with the vectors, the possibility remains that the viruses may have been carried only on the mouthparts and were washed off when the insects fed on healthy tissue. This latter interpretation is supported to a certain extent by results obtained by Fukushi (1939) who found that *Aphis laburni* loses the virus of red clover mosaic in feeding periods of 10 to 30 minutes on healthy plants but retains it for about an hour when it does not have access to food plants.

Mechanical transmission by contaminated mouthparts was suggested by Severin (1931) to explain rare cases of acquisition and transmission of the curly top virus by *Eutettix tenellus* in periods that may have been too short to permit the virus to pass through the insect.

If certain insects are able to transmit viruses by mechanical transfer on their mouthparts, they would probably be more effective in transmission of viruses that occur in parenchyma than of viruses restricted to phloem.

Where virus occurs in both phloem and parenchyma, it may be acquired from parenchyma, and infection may be produced by properly introducing it into parenchyma. Therefore, certain types of insects such as thrips, certain species of aphids and possibly other insects that feed chiefly or exclusively on parenchyma, are able to act as vectors. Even phloem-feeding insects may be able to function as vectors without actually feeding on phloem, as shown by Watson (1936) who found that *Myzus persicae* is able to acquire the Hy. III virus by feeding only on parenchyma of diseased plants and to introduce it into healthy plants in feeding intervals too short to permit the mouthparts of the insects to reach the phloem. In view of the possibility that phloem may be less favorable than parenchyma to multiplication of this type of virus, it would be of interest to have more information regarding the effectiveness of insect introduction of virus into parenchyma as compared with introduction into phloem.

Much evidence points to the conclusion that the vector relations of viruses that occur in both phloem and parenchyma are much less specific and limited than those of phloem-limited viruses. Drake, *et al.* (1933) showed that more than 50 species of aphids transmit the virus of yellow-dwarf of onion, and many other viruses of the mosaic type are known to be transmitted by more than one species of vector. Kenneth Smith (1937a) lists 21 viruses transmitted by *Myzus persicae*, 10 by *Macrosiphum gei*, and 8 by *M. pisi*. With few exceptions, the viruses transmitted by these insects produce mottling or local lesions. The probability of infection following introduction of virus into either phloem or parenchyma may be an important consideration in the production of these results.

RATE OF MOVEMENT

The rate of invasion of tissues by viruses following their introduction into the plant is influenced by a number of factors, the most important perhaps being: (1) the kind of plant in which movement occurs, (2) the kind of tissue in which movement takes place, and (3) the virus involved. Since the significance of these factors may vary in different plants and since other factors may exert an influence, a wide variation in the rate of movement among viruses may be expected. This expectation is abundantly realized in the results of a considerable number of measurements of virus movement that

have been made. As shown in table 1, these rates range from .18 cm. per hour, for one of the mosaic viruses of tomato, to 152 cm. per hour for the virus of curly-top of sugar beet.

The accuracy with which movement may be measured varies with different viruses. Accurate measurement of the rates of movement of mosaic viruses through the phloem is difficult because it is usually necessary to introduce these viruses into superficial cells, and entrance into the phloem is effected only after a period of relatively slow and variable movement through parenchyma. However, in tobacco the common mosaic virus is able to move as fast as 36 inches in 72 hours or at a rate of one-half inch per hour. The time interval includes the period of slow movement through leaf palisade and mesophyll and the rate of movement in the phloem is probably somewhat higher than that indicated. In tomato Kunkel (1939) estimates that this virus may move as fast as 7 inches per hour.

The movement of some of the leafhopper-transmitted viruses

TABLE I
RATES OF MOVEMENT OF VIRUSES IN PLANTS

Disease caused by virus	Plant in which movement occurred	Distance traveled in indicated time	Rate of movement; centimeters per hour	Reference
Mosaic	Tomato	8-18 inches in 10 to 15 days	1 to 2*	McCubbin and Smith (1927)
Mosaic	Raspberry	49 inches in 10 days	.52	Bennett (1932)
Curly Top	Sugar Beet	7 inches in 30 minutes	38.1	Severin (1924)
Curly Top	Sugar Beet	6 inches in 6 minutes	152.4	Bennett (1934)
Curly Top	Tobacco	24 inches in 48 hours	1.27	Bennett (1934)
Mosaic	Tobacco	13 cm. in 2 days	.29	Böning (1928)
Mosaic	Tomato	9 cm. in 2 days	.18	Böning (1928)
Mosaic	Tomato	14 inches in 2 hours	17.8	Kunkel (1939)
Streak	Maize	40 cm. in 2 hours	20.0	Storey (1928)

* Estimates given by McCubbin and Smith.

through the phloem can be measured with considerable accuracy, since the vectors are able to introduce the viruses directly into the vascular tissue. The viruses that have been found to move most rapidly are those of streak-disease of maize and curly-top of sugar beet. The virus of streak actually moved a distance of 40 cm. in 2 hours in a maize leaf and the virus of curly-top moved 6 inches in 6 minutes in a beet leaf. These movements are considered to have taken place in the phloem.

Some indication of the influence of the plant on rate of movement may be gained by comparing the movement of the virus of curly-top in sugar beet and in Turkish tobacco. In beet the virus moved 6 inches in 6 minutes or at a rate of 60 inches per hour and in tobacco it moved 24 inches in 24 to 48 hours or at a rate of not more than one inch per hour. Thus the virus moved at least sixty times faster in beet than in tobacco.

PATH OF VIRUS MOVEMENT

Virus activities such as multiplication and spread are believed closely linked with the chemical and physical processes that govern the functioning of living protoplasm. Within parenchyma cells, viruses appear to be closely associated with the cytoplasm. Livingston and Duggar (1934) concluded that the virus of tobacco mosaic probably is much more highly concentrated in the cytoplasm than in the vacuole. More recently Martin and McKinney (1938) found comparatively little virus in vacuolar sap and concluded that the cytoplasm contains most of the virus present in living tissue. This evidence, although not conclusive, indicates that cytoplasm is the chief path of movement of viruses in parenchyma cells.

Up to the present time, no evidence has been presented showing that any virus is capable of passing directly through the cellulose structure of the cell wall itself. Apparently infection does not take place through uninjured root hairs, trichomes, or other epidermal cells. The apparent inability of viruses to pass out of or into tracheae has already been mentioned. However, walls of tracheal and epidermal cells are somewhat specialized and perhaps would be expected to offer more resistance to virus passage than walls of certain other plant cells.

A certain amount of evidence is available on the resistance offered to virus passage by walls of cells within the leaf structure. By sub-

merging parts of detached leaves of *Nicotiana glutinosa* in liquids containing virus of aucuba mosaic, and then reducing the pressure of the surrounding medium, Caldwell (1932) succeeded in introducing virus into intercellular spaces of the mesophyll. Local lesions characteristic of the disease developed on leaves treated in this manner in only a few instances and these lesions were attributed to accidental infection through injuries. The treated leaves were shown to be susceptible to infection when the virus was introduced through wounds. It was concluded, therefore, that the virus was unable to enter the cells from intercellular spaces by passage through the unbroken cell walls.

In other work bearing on this subject, Duggar and Johnson (1933) reported infection with tobacco mosaic virus through stomata of tobacco leaves. It was suggested that when virus suspensions were sprayed on leaves virus particles passed through the stomatal openings and entered the protoplasts from the substomatal cavities. The question was raised, however, as to whether the virus might not pass from the intercellular spaces into the cells through protoplasmic fibrils. Sheffield (1936a) was unable to verify these results and suggested that the infection obtained did not take place through the stomata but resulted from accidental infection through wounds made in the process of inoculation.

If viruses are unable to pass through the cellulose structure of all types of cell walls, the path of movement from cell to cell is limited to protoplasmic connections between cells. Plasmodesmata have been suggested by a number of investigators (Quanjer, 1931; Livingston, 1933; Drake *et al.*, 1934; Sheffield, 1936; and Martin and McKinney, 1938) as avenues of virus passage from one cell to another, and much evidence seems to support this concept.

If plasmodesmata are the sole avenues of virus travel through cell walls, as the evidence indicates, the implications are rather interesting. In view of the large number of plants in which it is known definitely that viruses invade at least most of the living cells of the ground tissue of the plant, it naturally follows that a general idea of the prevalence and distribution of plasmodesmata in plants can be obtained by observing the way in which virus invasions take place. This evidence supports the view of universal occurrence of plasmodesmata in flowering plants, and suggests also that plasmodesmata are well distributed throughout living tissues and serve as effective avenues for passage of materials from cell to cell.

The phloem as a path of virus movement was first suggested by Beijerinck (1898). Others (see Henderson Smith, 1930) have since presented evidence supporting this view. More recent work on rates of movement and on the restriction of virus passage by rings breaking phloem continuity, leaves little doubt as to the importance of phloem as an avenue of virus travel.

If the path of virus movement is the cytoplasm in the ground tissue of the plant, it might appear to follow that cytoplasm functions also in the movement of virus in the phloem. This may be true to a limited extent. It seems reasonable to expect that viruses that move in cytoplasm of cells of epidermis, palisade, and mesophyll, for example, would move in the same medium in other cells of these general types, and, therefore, would move in the cytoplasm of the phloem parenchyma.

However, there is no reason to suspect that viruses would move more rapidly in the parenchyma of the phloem than in other types of parenchyma. Rates of movement through the agencies available in parenchyma are not sufficient to account for the observed virus movements in the phloem, and the directional movements are opposed to this concept. For these reasons it may be assumed that cells other than parenchyma are the avenues through which the rapid movements take place. It seems probable that these cells are the sieve tubes.

A more extensive knowledge of the factors involved in the movement of organic substances in general through the phloem would undoubtedly provide a basis for a clearer concept of the possible path of viruses through this tissue. But if the speed and direction of movement of viruses are considered in connection with the theories that have been proposed to explain transport of organic materials through the phloem, the possibility must be considered that viruses may be released from the cytoplasm when they pass into sieve tubes and may occur more or less free in the lumen of the sieve tube. This concept is supported by the fact that the curly-top virus occurs in high concentrations in the phloem exudate from sliced surfaces of beets and also in the phloem content that moves from the phloem through intercellular spaces of cells of adjacent tissues and appears as drops on the surface of beet leaves and petioles. It is not probable that this exudate contains solid constituents from the phloem, and therefore the virus that the liquid exudate contains

must have been free of the cytoplasm. In light of this evidence it seems probable that viruses occur free of cytoplasm in the phloem and that the chief path of rapid movement through phloem is the lumen of the sieve tube.

TISSUES IN RELATION TO SEED TRANSMISSION OF VIRUSES

Despite the fact that viruses are transmitted through the seeds of bean, wild cucumber, certain varieties of muskmelon, *Datura*, tobacco, lettuce, and potato, absence of seed transmission in general is very striking. This condition is not surprising with viruses that are restricted to the phloem. For, since there is no direct vascular connection between the embryo and the mother plant, the meristematic and parenchymatous tissue enveloping the embryo would function as an effective barrier to passage of virus in all stages of development of the embryo.

Lack of seed transmission of such viruses as that of tobacco mosaic, where invasion of the greater share of the living tissue occurs, is much more difficult to explain. Allard (1915) showed that the virus of tobacco mosaic passes into the ovule and occurs in both immature and mature seeds, but emphasized the fact that "A very efficient barrier guards against embryonic infection or the subsequent successful continuation of the disease from parent to seedling." Duggar (1930) found that ground seeds, especially those high in protein content, produced inactivation of the virus of tobacco mosaic but inactivation was never complete at the concentrations of proteins used. It was concluded that inactivation was not a factor of absolute protein content of the seed but was probably dependent on specific proteins or on specific compounds accompanying them. It was suggested that a probable relationship exists between lack of seed transmission and adsorption and inactivation of the virus by stored proteins in the seed. Lack of seed transmission under these conditions might involve entrance of the virus into the seed followed by inactivation by storage compounds.

Other evidence indicates, however, that lack of seed transmission may not depend on inactivation by the seed but on inability of virus to enter or remain active in such structures as microspores, megaspores, embryo sacs, and embryos. Much evidence points to the conclusion that seed transmission is determined at the time pollen and ovules are developing.

Nelson (1932) states that seeds from plants infected after flowering rarely transmit the virus of bean mosaic, indicating that virus transmission is determined in the very early stages of seed development.

Blakeslee (1921), working with the "Q" disease of *Datura*, found that diseased plants fertilized by pollen from healthy plants and healthy plants fertilized by pollen from diseased plants, produced seeds that transmitted the virus to the next generation, indicating the presence of virus in both pollen and ovules.

Reddick (1931) presented evidence indicating that the virus of bean mosaic is transmitted through the pollen of bean. Nelson and Down (1933), through cross-pollination studies in bean, found that about 25 per cent of the pollen grains from infected plants and about the same number of ovules from infected plants, carried the bean mosaic virus.

The yellow and green ringspot viruses of tobacco have been shown by Valteau (1932, 1939) to be transmissible in the seed. Each of these viruses causes varying degrees of pollen sterility and gives evidence of being present in the pollen. Valteau (1932) suggested that if a virus enters the pollen it may also enter the embryo sac and that, therefore, pollen deformities produced by viruses may have some significance in indicating probable seed transmission.

Reddick (1936) presented evidence suggesting that the seed-borne virus of acropetal necrosis of potato may be carried in the pollen of infected potato plants.

In reasonably extensive tests² no virus was obtained from pollen taken from beet plants infected with beet mosaic nor from pollen from Turkish tobacco plants infected with common tobacco mosaic. Neither virus is seed transmitted.

Further studies are needed to determine the relation of other viruses to pollen and to ovules of susceptible species of plants. It is significant, however, that there appears to be no definite record of pollen infection by a virus not seed-borne. Conversely, where investigations have been reported, viruses that are seed transmitted show evidence of being present in pollen. If this apparent correlation between pollen infection and seed transmission has a general application, as the somewhat limited evidence indicates, pollen infection may be an index to susceptibility of gametes and may point to

² Unpublished data.

the critical period of development during which seed transmission is determined.

The factors governing seed transmission of viruses may reside in certain tissues that give rise to the male and female gametophytes or in the gametophytes themselves. Thus the mechanism which in many plants affords protection to the succeeding generation probably is closely associated with the reproductive processes. This protective mechanism is not clearly evident but enough information is available to afford a basis for further speculation regarding its nature.

If virus is absent from meristematic tissues the megaspore mother cell would be expected to be free of virus as would the resulting megaspores. In the degeneration of three megaspores and the enlarging of the fourth to form the embryo sac, it is possible that crushing or degeneration of cells immediately surrounding the megaspore may destroy protoplasmic connections between embryo sac and adjacent cells. If protoplasmic connections are essential for movement of viruses from cell to cell, as seems probable, destruction of these connections would tend to prevent infection of the megagametophyte.

The escape of pollen grains from infection, however, could not be explained on this basis since four pollen grains develop from each mother cell and there is no evidence that breaking of protoplasmic connections between cells would take place in the early stages of pollen differentiation. However, it seems within the limits of possibility that differentiation and maturation may be so rapid that pollen grains may separate from the mother plant and from each other often before viruses have a full opportunity to enter them.

A second point worthy of consideration in this connection is the fact that the microspores and megaspores are structures of the gametophytic generation which may conceivably modify their reaction to viruses. Factors associated with the production of sporophytic and gametophytic generations are capable of producing enormous morphological changes, as witnessed by the form differences between the sporophytic and gametophytic generations of plants of such groups as liverworts, mosses, and ferns. Morphologic differences of these magnitudes must be the result of distinctly different chemical constitutions and physiological functionings.

It seems logical to expect that in addition to the morphologic differences exhibited by individuals of the two generations, other

differences equally marked would result and that some of these differences might well involve susceptibility to attack by viruses and parasitic organisms. The gametophytic generation of higher plants may contain protoplasm essentially different from that of sporophytic generation in respect to its resistance to invasion by viruses. Therefore, invasion of the gametophytic generation of a plant by a virus present in the sporophytic generation would be somewhat a matter of chance and on this basis probably would not be expected to occur oftener than infection of plants selected at random from the general population of species of the groups of plants attacked by the particular virus in question.

If a virus failed to enter the megagametophyte or microgametophyte for these reasons, or for other reasons, fertilization would initiate a virus-free embryo in a virus-free medium. The embryo, as a rule, develops rapidly, and it seems doubtful as pointed out by Caldwell (1934) and by Sheffield (1936) that there are any protoplasmic connections between the young embryo and the adjacent cells in any stage of embryonic development. The rapid elongation of the embryo in most instances would tend to break protoplasmic connections with adjacent cells if any were formed. In the absence of such connections it does not seem probable that infection of the embryo by passage of virus from adjacent cells into the embryo would be likely to occur even with viruses that are capable of reaching appreciable concentrations in the tissues immediately surrounding the embryo.

On the other hand, if a virus were able to enter the embryo sac or the microgametophyte and remain active, fertilization would result in the initiation of an embryo in a medium containing virus. The virus would be expected to remain in the cytoplasm of the zygote and its derivatives when cell walls were laid down, and would pass on to other cells in the succeeding cell divisions in the growth and development of the embryo, and become seed-transmitted.

The improbability of embryo infection by direct passage of virus from adjacent cells together with the apparent correlation between pollen infection and seed transmission in the instances cited, indicate that seed transmission may hinge on ability of virus to enter the megaspores, microspore or embryo sac and maintain itself in these structures through their successive developmental stages and through the developmental stages of the structure resulting from the fertilized egg.

CORRELATION BETWEEN VIRUS MOVEMENT AND
FOOD TRANSLOCATION

During the past few years considerable evidence has accumulated that indicates quite strongly that the movement of viruses through infected plants is closely correlated with transport of organic food materials. This evidence has recently been reviewed by Crafts (1939).

In some of the earlier work on this subject it was shown (Bennett, 1927) that the virus of leaf-curl of raspberry produced symptoms on the top of the inoculated cane and moved downward into the root system, but under normal conditions it did not produce symptoms on non-inoculated canes during the first season. It was induced to move into non-inoculated canes, however, by cutting them back or by removing their leaves. The spring following the season of inoculation, the virus moved into all of the canes not previously invaded.

Results of experiments in which non-inoculated canes were ringed at intervals before and after growth started in the spring, indicate that the virus moved into these canes when the lateral shoots were about 2 to 4 inches long. It was suggested that failure of the virus to move into non-inoculated canes during the season of inoculation was due to the inability of the virus to move counter to the direction of major transport of organic materials, and that movement into these canes the season following inoculation was associated with the movement of food reserves from the infected root system following depletion of the food reserves in the canes.

Studies on the movement of the virus of tobacco mosaic have given results from which conflicting conclusions have been reached.

Holmes (1931) found that from the point of introduction of the virus into a tobacco leaf, the virus moved more rapidly in the direction of the petiole than in the direction of the leaf periphery. In later work he (1932) found that shading inoculated and non-inoculated leaves induced changes in the path of movement that indicated some direct or indirect connection with the carbohydrate supply. However, since the virus was able to move from leaves starved with respect to carbohydrates it was suggested that the relation may be indirect.

In experiments with tomato, Samuel (1934) found that after being restricted to a limited area of the inoculated leaf for a period

of 2 to 4 days following inoculation, the virus moved rapidly toward the root system, then upward to the top of the plant except in plants with fruit clusters, in which case it moved into the fruits first. Samuel suggested a direct correlation between food movement and virus movement, and postulated, on the basis of his evidence, that the metabolites from the leaves moved first to the roots and then to the tops. More recent work by Kunkel (1939) has shown, however, that when the virus of tobacco mosaic passes from an inoculated leaf into the stem of a tomato plant, movement is not always first in the direction of the roots but may be either upward or downward or in both directions from the point of entrance.

Grainger (1933), by inoculating tobacco leaves at the distal ends and severing them at different distances from the point of inoculation at different time intervals, obtained results which he interpreted as indicating that the virus of tobacco mosaic moved at a uniformly accelerated rate. He concluded that movement was through the ground tissue and that it was associated with multiplication of the virus and unrelated to food translocation.

Caldwell (1934) found that the virus of tobacco mosaic passed out of leaves that were inoculated and placed immediately in the dark, and that the virus showed no greater tendency to enter mature leaves in the dark than it did to enter mature leaves in the light. Movements in the stems were in two directions. He (1936) found also that the virus was able to move out of immature leaves. Caldwell (1934) concluded that virus movement is independent of movement of food materials and that under certain conditions movement was apparently in a direction opposite that of the metabolites.

However, the results obtained by Grainger and Caldwell are capable of interpretations that do not necessarily conflict with the concept of a correlated virus movement and food translocation. As Caldwell pointed out, tobacco leaves wilt and die after they have been in the dark for about ten days. It is possible in such leaves that the directional movements of organic materials are not greatly different from those in normal leaves, due first to an outward movement of reserves and later to outward movement of products of protoplasmic disintegration.

Movement of virus out of immature leaves may be correlated with outward movement of organic materials during periods favorable for rapid carbohydrate synthesis. It is of interest in this connection

that Holmes (1932) found a greater movement of virus toward the periphery in immature leaves than in mature leaves and considers that "The reversal of the direction of movement of virus in leaves inoculated when very young seems to indicate a relation of some kind between the movement of food into a young and dependent leaf and the movement of virus, and suggests that virus moves toward the periphery until the leaf reaches a degree of maturity which allows it to export some food material to dependent growing parts."

Although it has been shown repeatedly that the virus of tobacco mosaic moves from an inoculated leaf to both the top and the roots in Turkish tobacco in a relatively short period, it has not been shown that movement in the two directions is simultaneous. Diurnal or other directional reversals of food movement would account for the observed virus movements.

In more recent work (Bennett, in press) additional evidence supporting the concept of a correlation between the movement of the tobacco mosaic virus and food translocation has been obtained. In vegetative plants of Turkish tobacco having a main stem in a horizontal position and a basal sucker in a vertical position, basipetal movement was rapid and acropetal movement was slow. The reverse was true in similar plants maturing seeds on the main stem. In vegetative plants, acropetal movement was accelerated by darkness and defoliation. Basipetal movement was very slow in main stems in the dark and in the majority of plants tested the virus failed to move out of darkened stems in 40 days; whereas in comparable stems in the light it moved out in all instances in 4 days or less.

In plants of *Nicotiana glauca* having top and basal grafts of Turkish tobacco separated by 3 feet of stem, virus moved from the top graft to the basal graft and produced symptoms in 6 to 9 days. Movement in the opposite direction, presumably counter to the direction of major food transport, was very slow and in some instances the virus did not move a distance of 3 feet acropetally in periods of 224 to 253 days.

McClean (1931) states that the virus of bunchy-top of tomato is restricted to leaves partially developed at the time of infection or developed subsequent to infection. When only the roots of tomato plants were inoculated no symptoms developed on the top. It is

possible that this latter result may be due to inability of the virus to move out of the roots against a food gradient, rather than inability of the virus to produce infection through root tissue.

It is probable that certain parts of root systems of plants may be able to localize viruses for considerable periods under normal conditions of growth. Mulvania (1930) found that when roots of tobacco plants were inoculated with the virus of tobacco mosaic, symptoms did not appear on the tops of the inoculated plants. Bennett (in press) obtained similar results but found that the roots were susceptible to infection and that the virus could be induced to move out of the root system by removing the tops.

Kunkel (1930) found that when peach buds from yellows trees were placed in vigorous young peach trees some distance above the ground level symptoms of yellows developed in a minimum time of six weeks; whereas when the infected buds were placed at or near the ground level the incubation period of the disease often extended over many months. This was found to be due to the fact that the virus moves quickly down but rather slowly up the peach stem. Downward movement was estimated to be about 10 times faster than upward movement. On a basis of a correlated virus and food movement these results would be expected except perhaps when the trees were producing rapid top growth immediately following a period of dormancy.

In studies (Bennett, 1937) on the movement of the virus of curly-top of beet it was shown that the virus moved downward from grafts of Turkish tobacco through stems of *Nicotiana glauca*, much more rapidly than it moved in the opposite direction. Defoliation of the tops, however, stimulated rapid movement upward.

Using the same virus it was found that in beets with three shoots on a common root system, the virus was retained in an inoculated shoot for periods as long as six months with no appearance of virus in non-inoculated shoots during this time. However, defoliation of one of the non-inoculated shoots resulted in movement of virus into it and the production of symptoms in a period of a few days. Also, when one of the non-inoculated shoots was placed in the dark it soon became diseased. A repetition of this experiment using the virus of beet mosaic gave similar results.

A relatively high concentration of curly-top virus was found (Bennett and Esau, 1936) in seeds from infected plants, perhaps

indicating a movement of virus into seeds with food materials, resulting in its accumulation as a residue.

The virus moved inward from the point of inoculation at the tip of a green leaf of sugar beet a distance of 6 inches in 6 minutes, whereas in etiolated leaves in the dark it failed in most instances to move out of the inoculated leaf in periods of 7, 14, and 21 days. These results were interpreted as indicating, not only that there is a correlation between virus movement and food translocation, but also that virus movement is dependent largely on the same agencies that are responsible for food translocation.

The available evidence favoring the view that virus movement is correlated with translocation of organic solutes seems extensive enough to justify serious consideration of the possibilities of using viruses as indicator materials in the study of the general subject of translocation in plants. If viruses may be used for this purpose they have the following distinct advantages: (1) If viruses are high molecular weight proteins and if they are produced from normal plant proteins as suggested by Stanley (1936), they may not be greatly different in many basic chemical and physical properties from certain normal protein constituents of the plant. Movement, therefore, would be expected to closely parallel that of the parent proteins. (2) Viruses may be introduced into epidermal cells by rubbing inoculum over the surface of leaves, or directly into the phloem by utilizing a suitable insect vector. Thus by selection of the virus and the method of introducing it into the plant, movement in either ground tissue or phloem may be studied. (3) Viruses move and remain active in a very large number of plants, giving a wide range of species and varieties for study. (4) The extent of spread from points of introduction may be determined by several different methods some of which permit a high degree of accuracy of measurement.

The objection has been raised (Curtis, 1935) that since viruses cause phloem necrosis and other abnormalities their movement may be abnormal and unrelated to normal solute movement. It may be pointed out, however, that the majority of viruses cause no detectable injury to parts that are mature at the time of virus introduction. It is in such parts that virus movement can best be studied. Moreover, few of the viruses that cause mottling produce detectable injury to the phloem, and it is not difficult to find viruses that cause

no recognizable symptoms on certain species and varieties of plants. The pathologic effects, therefore, would not seem to offer any great difficulties in the use of viruses as indicators of movement.

MECHANICS OF VIRUS MOVEMENT

It seems highly improbable that viruses possess any autonomous means of locomotion, since virus particles are known to be very small and much evidence indicates that they may be of molecular proportions. Movement, therefore, must result from the operation of ordinary physical and chemical forces common to the plants in which the viruses occur.

Evidence indicates that viruses are subjected to forces that produce two distinctly different types of movement, as indicated by the respective rates of travel. One set of factors gives rise to a relatively slow movement through parenchyma, and a second set of factors gives rise to rapid movement through the phloem. These two types of travel may be considered separately.

Movement in parenchyma. Uppal (1934) made measurements of the spread of the virus of tobacco mosaic from the upper epidermis to the lower epidermis in leaves of *Nicotiana glauca* and found a movement of 7 to 8 microns per hour. For a number of other viruses the rate of movement through parenchyma can be rather accurately estimated by measuring the rate of radial spread of local lesions. Measurements of this type with tobacco mosaic, beet mosaic, tomato spotted-wilt and bean mosaic have given a relatively uniform result and have indicated a radial spread of less than 1 to about 2 millimeters per day. Other evidence indicates that spread of viruses in general through parenchyma is of this order. Therefore, factors responsible for this movement probably need not account for movements much in excess of 2 millimeters per day.

In the movement of the virus of common tobacco mosaic from cell to cell, Stanley (1936) suggested that active virus protein in one cell may catalyze the production of virus protein in adjacent cells without actually passing out of the cell. Under such conditions movement of virus from cell to cell would not necessarily occur but would only appear to take place due to the catalytic action of virus protein at the periphery of the protoplasm of one cell on the precursor protein in adjacent cells.

However, it does not seem necessary to invoke the operation of such a mechanism in explaining movement from cell to cell. Intro-

duction of the virus into a living parenchyma cell would be followed by distribution of the virus to all parts of the cell by protoplasmic streaming and diffusion. The virus would readily pass into adjacent cells through the plasmodesmata by means of diffusion, perhaps aided by protoplasmic movement. Multiplication of virus probably would influence the rate of spread by diffusion to a certain extent, but it would be expected that maximum concentration would soon result at the point of introduction and that on the advancing margins of virus invasion a state of equilibrium would be reached in respect to concentration and diffusion and that soon after introduction the virus would reach a state in which it would move at a uniform rate, in so far as rate of movement was affected by diffusion, and not at a uniformly accelerated rate as has been suggested for certain viruses.

Movement in phloem. The rapid rates of movement of viruses in phloem (as high as 1 inch per minute) indicate that protoplasmic streaming and the ordinary rates of diffusion may be ignored as major factors in accounting for this movement. The apparent correlation with food translocation indicates that movement through the phloem may be dependent on normal processes that function in plants in the transport of food materials. For this reason it may be well to consider some of the mechanisms that have been proposed to explain the transport of various kinds of substances through plants, in connection with the probable mechanism responsible for virus movement.

The rates of movement of the viruses of streak of maize (.3 cm. per minute) and of curly-top of sugar beet (2.5 cm. per minute) are so great that they may suggest a movement similar to that of a stimulus. These rates are more or less of the same order, however, as those calculated for movement of sugars through the phloem of potato, cucurbits (Crafts, 1931, 1932), and cotton (Mason and Maskell, 1928). These results with the movement of sugars demonstrate that the plant possesses a mechanism capable of effecting rapid transport of elaborated materials. There seems no logical reason, therefore, for assuming that virus invasion of the phloem network is not brought about chiefly by actual transport of virus units.

Went (1932) suggested that growth hormones move along electrical potentials toward rapidly growing regions. It has been shown

that, in general, virus entities, or the particles to which they are attached, carry a negative charge. It is conceivable that a positive potential might cause rapid movement of virus particles under certain conditions. However, to accept this theory of virus movement certain assumptions regarding conditions and changes in plants must be made. For example, it would be necessary to assume that the potential is much less effective in causing movement in parenchyma than in phloem. In the case of the virus of curly-top it would be necessary to assume that the potential gradient is basipetal in stems of *Nicotiana glauca* and in the beet leaf and that defoliation or darkness is capable of reversing this gradient.

Van den Honert (1932) called attention to the possibility of movement of materials along the interfaces of protoplasmic material in the phloem due to surface tension forces. Possibly such a mechanism would provide for the rates of virus movement observed if viruses are capable of moving as monomolecular films along the surface of protoplasmic layers, but it would not provide for the directional movements observed. In this system substances would move independently and equally in all directions in which there were paths for movement. Therefore, viruses would move against food gradients as fast as they move with food gradients, and defoliation and darkness should have no effect on rate of invasion of a plant part, all of which is opposed to the observed facts.

One of the oldest theories of food movement invokes diffusion and protoplasmic streaming to bring about transport. Curtis (1935) has reemphasized this theory with the suggestion that protoplasmic streaming in the sieve tubes may occur and possibly may be much faster than heretofore suspected. However, even if streaming of protoplasm in the sieve tube actually occurs, it does not seem probable that it can be fast enough to provide for the more rapid rates of virus movement, and so far as viruses are concerned the theory has the added weakness that it falls far short of accounting for the directional movements observed.

Münch (1930), in a theory that was later discussed and modified by Crafts (1931), proposed that there is a pressure flow of liquid materials through the phloem from supplying to receiving cells. According to this concept, sugars pass from the synthesizing cells into the phloem where an increase in osmotic pressure is produced resulting in a higher hydrostatic pressure. This causes a move-

ment of materials through the phloem in the direction of regions having lower hydrostatic pressures. The osmotic pressure is lowered in regions of utilization and storage by removal of sugars from the phloem. This results in loss of water by the phloem and in reduction of hydrostatic pressure in these regions. Movement, therefore, toward regions of utilization and storage would be continuous.

The major objections to this theory of transport are summarized by Curtis (1935). He suggested that perhaps the exudate which is obtained from the cut ends of cucurbit stems and which has been assumed to flow from the phloem, does not originate in the phloem and therefore cannot be accepted as evidence of flow through the phloem of the uninjured plant. He suggested further that the theory of pressure flow as modified by Crafts rests on at least three unproved assumptions: (1) that the supplying cells can in some way introduce sugars into the phloem in such a manner that they will develop a pressure gradient leading to the receiving cells, (2) that the sieve tubes are completely permeable and offer a minimum of resistance to the flow of solutions through the lumina and cross sections walls at all points and yet that the phloem is so enclosed by cambium and phellogen as to prevent leakage, and (3) that receiving cells can absorb sugars against a gradient with such rapidity as to lower greatly the concentration within the walls outside the living membrane. The general theory of mass flow of solutes in the phloem is criticised because it does not allow for simultaneous movement of materials in two directions.

Upon critical analysis, however, and in the light of a certain amount of additional evidence, these objections do not seem to constitute insurmountable obstacles to the acceptance of the general principles of the theory of pressure flow.

Further work by Crafts (1936) furnishes strong support for the contention that the exudate obtained from the cut stems of cucurbits is derived directly from the phloem. Recently Crafts (1939) demonstrated exudation from the cut surfaces of plants of *Macrocystis* and states that this exudate unquestionably came from the phloem, since there is no xylem in *Macrocystis* and the sieve tubes of the phloem are the only specialized elements capable of rapid conduction. It was stated also that by using suitable technique phloem exudation can be demonstrated quantitatively in most woody species.

These results seem to show rather conclusively that liquids are able to flow through cross-sectional areas of the phloem under a positive pressure.

Phillis and Mason (1933) showed (as Curtis noted) that the phloem of the cotton plant is able to accumulate sugars against a gradient. This suggests at least that a mechanism may exist in the plant for establishing pressure gradients in the phloem.

Whether the resistance which the phloem offers to mass flow of materials is prohibitive remains to be determined. An earlier idea (Crafts, 1931) that movement takes place chiefly through the cell walls of the sieve tubes seems untenable, as pointed out by Steward and Priestley (1932), because of the high pressures required. However, calculations by Crafts (1933) indicate that if the sieve tube lumina are the chief channels of transport the pressure required may not be excessive. The degree to which the cell layers incasing the phloem are able to preserve a positive pressure in the phloem cannot be stated with certainty, but the mere fact that positive pressures exist in the phloem shows that the encasing layers are able to function with at least a certain degree of effectiveness in this respect.

The rapidity with which receiving cells can absorb sugar from the phloem and thus steepen the sugar gradient in the phloem undoubtedly varies considerably with conditions. It is evident that such plants as sugar beet, sugar cane, and date are able to remove sugar from the phloem and accumulate it in storage cells against steep sugar gradients. The rate of storage in some of these plants shows that movement from the phloem into the receiving cells is rapid.

It is quite obvious, of course, that the pressure flow concept does not permit of continuous simultaneous two-directional movement through the phloem. More evidence is needed as to the amount of two-directional movement necessary to satisfy the plant's distributional requirements, and as to the extent to which such movement actually occurs. The evidence for simultaneous two-directional movement is limited. Phillis and Mason (1936) found that nitrogen and carbohydrates moved simultaneously in opposite directions in the stems of cotton plants. Although they consider that in their experiments movement of both nitrogen and carbohydrates probably took place in the phloem, they point out that the possibility

that the nitrogen moved in the xylem cannot be excluded. Palmquist (1936) presented evidence interpreted as indicating simultaneous movement of carbohydrates and fluorescein in opposite directions in the phloem of bean leaves. There is still some question, however, as to the tissue in which fluorescein moves and also as to the factors involved.

It would seem that further effort should be directed toward determination of the possibilities for differential distribution of materials that may be effected by frequent directional reversals of movement of materials and toward determination of the extent of movement of materials in opposite directions in different vascular bundles of the same stem or leaf.

Much of the evidence on virus movement strongly supports the concept of mass flow of liquid phloem content. This evidence clearly indicates that with certain viruses at least, movement is decidedly unidirectional from the point of introduction and movement is in the direction of major transport of elaborated food materials. The evidence for correlation between virus movement and food transport has already been summarized. It seems to clearly indicate that viruses move rapidly in directions of food utilization and storage, and slowly in opposite directions. A correlation of this kind would indicate some type of flow through the phloem.

Virus movements that may be interpreted as furnishing doubtful support to the pressure flow concept involve a type of possible two-directional movement and apparent differences in rates of movement of two viruses introduced into the plant simultaneously.

Evidence for two-directional movement of tobacco mosaic in tomato was presented by Kunkel (1939). In these experiments the virus was allowed to enter the stem of tomato plant through the petiole of an inoculated leaf located approximately midway between the top and roots. In a two-hour period the virus moved only to the tops in some plants, only to the roots in other plants, and to both tops and roots in still other plants.

Kunkel considers that the virus moved simultaneously in two directions in the stem in some of these plants, but suggests that movement in the two directions may have been in different vascular bundles. It would seem possible to have such a movement also in the same vascular bundle under certain conditions, if virus movement is influenced by food translocation. In a rapidly growing

tomato plant carbohydrates are being used by the top and by the roots. These carbohydrates are supplied by leaves located along the total length of the stem. Assuming demand by both top and roots there must be a portion of the stem in which movement is toward the root and a portion in which movement is toward the top. This would provide a stem zone out of which movement would be in two directions. The position of this zone would be expected to fluctuate with varying demand for food by top and roots. If virus were being introduced into this zone it might, in some cases, move in both directions from the point of entrance, and movement might be in one or more vascular strands.

The failure of the virus, moving at a maximum rate of 7 inches per hour in these tests, to move toward the root in certain plants in periods of 12 to 28 hours is decidedly opposed to the concept that the factors responsible for movement operate continuously to cause simultaneous movement in two directions in all parts of the stem.

Differences in the rate of invasion of plants by the separate components of a virus mixture have been found in certain instances. Smith (1931) found that when the "X" and "Y" viruses of potato are introduced into a leaf by mechanical inoculation, the "Y" virus appears in the young leaves of the inoculated plants about 2 days ahead of the "X" virus. Curtis (1935) pointed out that this seems opposed to the concept of unidirectional flow of phloem content. As Samuel (1934) has emphasized, however, it has not been demonstrated that two such viruses actually move at different rates in the phloem but only that they arrive at points of test at different times. This difference in time of arrival at points of test may result from failure of two viruses introduced simultaneously into the epidermal cells of leaves, to move at the same rate through intervening parenchyma cells and to enter the phloem at the same time. Unfortunately no results are available as to the relative rates of movement of viruses introduced simultaneously into the phloem instead of parenchyma.

It is recognized, however, that even if two viruses were introduced simultaneously into the same sieve tube they might move at different rates in a flowing medium if they differed in size or shape. There is considerable evidence indicating that the pores in the sieve plates are, in some plants at least, extremely small and filled with slime.

If materials move through these pores, or if they move partially through the cell walls as suggested by Crafts (1932), particles of different shapes and sizes may encounter different degrees of resistance to movement. Other things being equal, small spherical particles would encounter less resistance in passing through such obstructions than would be met by larger spherical particles or by elongated particles. Rate of movement, therefore, may be influenced to a certain extent by the size and shape of the virus particles and possibly also by their electrical charge.

Differential travel of components of virus mixtures from plant roots toward the tops of actively growing shoots has been found in certain instances and probably occurs with a number of virus combinations.

For example, when plants of certain susceptible varieties of raspberry were inoculated with the viruses of leaf-curl and red raspberry mosaic, simultaneously, it seems quite certain that the latter virus invaded the non-inoculated canes of the plant more rapidly than the same canes were invaded by the leaf-curl virus. When a cane of a black raspberry plant was inoculated with the viruses of yellow mosaic and red raspberry mosaic, the virus of red raspberry mosaic moved into the non-inoculated canes of the plant during the first season; whereas, the virus of yellow mosaic did not move into canes until the second season.

When a shoot of a beet plant having three shoots was inoculated (Bennett, 1938) with the viruses of mosaic and curly-top the virus of mosaic, as a rule, moved into the non-inoculated shoots several days or even weeks before the presence of the virus of curly-top could be demonstrated in such shoots.

It has not been shown in any of these experiments that the downward movement in the phloem from the points of introduction and in the direction of major food movement, was different for the components of the virus-combinations involved, but only that upward movement into non-inoculated canes or shoots, counter to the direction of major food movement, was different.

When the possible tissue relationships of these viruses are taken into consideration this evidence does not necessarily conflict with the idea that mass movement of materials in the phloem is important in the movement of materials throughout the plant. There is strong indication that the viruses of leaf-curl and curly-top are closely re-

stricted to the phloem and there is some evidence also that this may be true of the virus of yellow mosaic of raspberry. The viruses of red raspberry mosaic and of beet mosaic probably occur in both parenchyma and phloem. A differential movement of the type observed might be expected with two viruses having these two tissue relationships.

If they were introduced into the phloem of a leaf or the tip of a cane, simultaneously, both would move toward the root system at the same rate if they were carried by mass flow of phloem content, assuming no selective interference with movement. After entering the crown or root the virus restricted to the phloem would move more slowly upward through mature parts against a downward flow of materials and at times its movement backward due to mass flow might be faster than upward movement due to the operation of other factors.

The virus able to move and multiply in both parenchyma and phloem might effect much of its upward movement in parenchyma tissue. This movement would be relatively slow. Also, due to temporary changes in food relations, it might be carried upward by surges of materials from the roots, if liquids in the phloem move under a pressure gradient. Such movements would be expected to carry both types of virus upward in the phloem for the distance of the movement. The phloem-limited virus would remain in the phloem and tend to be carried back when the direction of movement of materials was reversed. The mosaic virus would be expected to pass out of the phloem into adjacent parenchyma tissue and become established there. From this newly invaded region the virus would not only continue to travel through parenchyma, but it would also multiply and furnish a more or less permanent source of virus to the phloem at the new levels of invasion. Each successive upward movement of materials in the phloem would elevate the virus to higher levels in the non-inoculated part.

It is easily conceivable that in this manner, two viruses with the indicated tissue relationships, could readily be separated, one passing into non-inoculated parts more rapidly than the other even though the two viruses were introduced into the phloem of the plant at the same point, simultaneously.

In this connection it is a rather significant fact that viruses such as the virus of red raspberry mosaic and the virus of beet mosaic,

that evidently occur in both phloem and parenchyma, travel in directions opposite major food movement at greater rates than viruses such as the virus of leaf-curl of raspberry and the virus of curly-top of sugar beet, that appear to be more or less restricted to the phloem. Rates of travel of viruses of the first types in directions opposite major food movements, however, are far below those determined for movement in the directions of major food movement.

Definite conclusions at this time regarding the mechanism responsible for virus movement through the phloem would be premature. From the evidence indicating a correlation between virus movement and food transport it may be suspected, however, that movements of both viruses and food materials are brought about by the same basic physiological processes. If this is true, the observed virus movements are opposed to the concepts of transport by diffusion and protoplasmic streaming and movement along protoplasmic interfaces but they harmonize reasonably well with the concept of pressure flow of liquid phloem content.

The existence of a plant mechanism capable of bringing about a pressure flow of liquid phloem content is still a matter of controversy among plant physiologists. It may be said, however, that if such a mechanism does operate it would satisfy most of the requirements of virus movements that have been determined. It follows, therefore, that the mechanism responsible for virus movement must be able to effect movements essentially similar to those that would be expected to result from pressure flow of liquid phloem content. For this reason the pressure-flow concept may be helpful to those interested in virus movement. On this basis a logical picture of invasion of plants by viruses may be drawn which conforms in all essential details with the virus movements that have been observed.

Under such a concept, the introduction of a mechanically transmissible virus into the epidermal cells of a leaf would be followed by increase, and distribution would be effected by protoplasmic streaming and diffusion perhaps aided by the processes responsible for virus increase. The virus would pass into adjoining cells, probably through plasmodesmata, and be distributed by the same processes that were responsible for distribution of virus in the inoculated cell. Eventually the virus would pass into the phloem through the protoplasmic connections and come in contact with a stream of material moving more or less rapidly toward regions of food utilization

or storage. Phloem-limited viruses would be introduced directly into the phloem content through the agency of insect vectors.

Viruses would tend not to move into mature leaves and regions supplying an excess of photosynthates. A reversal of food requirements in any region would cause a reversal of flow in the phloem, and virus would be carried passively in the direction of the new movement. In plants in which food movement can be modified and to a considerable extent controlled by defoliation and darkness, virus movement could be correspondingly influenced. This would explain the rapid movement of the curly-top virus into defoliated or darkened shoots of beet when corresponding shoots not defoliated or darkened remained free of virus for relatively long periods. It would account also for the apparent tendency of the virus of tobacco mosaic to move toward fruiting parts in tobacco and tomato and for the relatively high concentrations of curly-top virus found in beet seeds from infected plants. Etiolated leaves or shoots receiving food from regions of supply would be expected to retain virus as they have been observed to do in beet and tobacco.

SUMMARY AND CONCLUSIONS

In their increase and movement in infected plants viruses appear to be limited to living tissues. However, there is evidence that different types of living tissue offer varying amounts of resistance to virus invasion, depending on the plant affected, on the virus involved, and on the environmental conditions to which the infected plant is subjected.

The principal kinds of tissues known to be susceptible to virus invasion are the phloem and the various types of parenchyma. All types of meristematic tissue seem to be resistant. In some instances there is evidence that cell invasion at growing points may not occur until differentiation into parenchyma begins. If viruses occur in meristem, they apparently cause no direct injury to this type of tissue.

On the basis of their ability to invade parenchyma and phloem, viruses exhibit three more or less clearly defined relationships to tissues: (1) restriction to parenchyma, (2) close association with the phloem, and (3) occurrence in both phloem and parenchyma.

Viruses that are restricted to parenchyma are less common than those that show other relationships to tissues. This would be expected since such viruses would be less readily disseminated.

However, evidence suggests that the virus of phony disease of peach may be limited to parenchyma of the woody cylinder and it seems probable that the virus of tobacco necrosis may prove to be limited to parenchyma under most conditions. Certain normally systemic viruses are evidently limited to parenchyma in plants where they produce only local lesions.

Several viruses that appear to be rather closely restricted to phloem are known. Leaf-curl of raspberry and curly-top of sugar beet are considered representative diseases caused by viruses of this type. Phloem-restricted viruses produce diseases characterized by disturbances arising mainly in the phloem. Typical symptoms are phloem necrosis, vein translucency and distortion, leaf distortion and rolling, and general stunting and discoloration of the affected plant. Infection by mechanical inoculation is difficult. Vectors are limited to insects that feed on the phloem and probably also to insects that are able to pass virus through their bodies and liberate it with the saliva in feeding.

Viruses that occur in both phloem and parenchyma are numerous, and probably include all of the viruses of the mosaic-producing type. Characteristic symptoms result from disturbances in the parenchyma, and consist chiefly of local lesions and mottling. Phloem disturbances are usually minor or absent. Often infection by mechanical inoculation is easily accomplished. In general, insect vectors are more numerous and tend to lack specificity. Phloem-feeding is not essential to transmission.

The measured rates of virus movement following introduction into the plant vary from one-tenth of a centimeter per hour for the virus of tomato mosaic in tomato, to 152.4 centimeters per hour for the virus of curly-top in sugar beet. Rate of movement is influenced by the plant in which movement takes place. For example, the virus of curly-top moves at a much greater speed in sugar beet than in tobacco. The extent to which different viruses may move at different rates in the same plant remains to be determined. Evidence available at present indicates that certain factors operating in the plant may be more important than the virus in determining rate of movement.

The path of virus movement appears to be the living cell. Apparently viruses are unable to move through the cellulose structure of the cell wall. Limited evidence suggests that movement through

parenchyma takes place in the cytoplasm. Plasmodesmata probably serve as avenues of passage from cell to cell. When viruses enter the vascular elements there is indication that they are released into the liquid content of the phloem and it seems probable that the chief path of movement through the phloem is the lumen of the sieve tube.

In the absence of vascular connections between the embryo and the mother plant, restriction of a virus to the phloem would prevent seed transmission. Freedom from seed transmission of viruses that occur abundantly in parenchyma is more difficult to explain. It seems evident, however, that the factors involved are associated with the mechanism of reproduction. Possibly protection to the succeeding generation may be afforded by inability of viruses to enter microspores and megaspores and maintain themselves throughout the subsequent developmental stages of these structures and their derivatives. The fact that pollen infection is associated with all cases of seed transmission that have been investigated adequately, and that no virus not transmitted in the seed is known to occur in pollen, lends support to this hypothesis.

Two possible explanations may be advanced to account for failure of viruses to enter microspores and megaspores: (1) Destruction of protoplasmic connections between sporophytic tissue and the microspores and megaspores may take place before viruses have entered the latter structures; (2) The gametophytic generation may in many cases possess immunity to infection due to physiological characteristics resulting from its gametophytic constitution.

If virus failed to enter the gametophytic structures, fertilization would be expected to initiate an embryo in a virus-free medium. Since there is no evidence of plasmodesmatal connections between the embryo and adjacent cells, the embryo would be expected to remain free of virus even though adjacent tissue might be infected.

Certain viruses have been shown to move more rapidly in directions of regions of food utilization and storage than in opposite directions. The direction of major virus movement may be partially controlled by influencing the direction of food transport by such measures as reducing the leaf surface on appropriate plant parts or by subjecting the parts to prolonged periods of darkness. Rather extensive evidence supports the concept that virus movement is definitely correlated with the normal transport of organic food materials.

Factors responsible for invasion of plants by viruses must provide for two distinct types of movement. The first is a slow movement through parenchymatous tissues and the second is a much more rapid movement through the phloem.

Movement through parenchyma is probably effected through protoplasmic streaming and diffusion possibly aided by the processes responsible for virus increase.

The factors responsible for movement of viruses in the phloem are of considerable interest because of the speed and directions of movement in this type of tissue. These factors must provide for movements as great as 2.5 cm. per minute and for a type of unidirectional movement which seems to be correlated with transport of photosynthates. It seems probable on the basis of rate and direction of observed virus movements, that diffusion, protoplasmic streaming, forces operating to promote movement along protoplasmic interfaces, and possibly electrical potentials, may be ruled out as major factors in the movement of viruses in the phloem. In light of present knowledge it seems probable that the mechanism responsible for virus transport in the phloem is able to effect movements essentially similar to those that would be expected to result if a pressure-flow mechanism, such as that proposed by Münch (1930), were operating in the transport of elaborated food materials.

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MEDICAL MYCOLOGY

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Pathogenic fungi were among the first microorganisms to be recognized as etiologic agents of disease. Schoenlein in 1839 observed and described the fungus causing favus, and in the same year Lagenbeck described the fungus which causes thrush. Gruby independently discovered these two fungi, and in 1843 the fungus of tinea tonsurans. More exact information about these and other pathogenic fungi followed the studies begun in the last decade of the 19th century by Sabouraud, Rixford, Gilchrist, Schenck, Buermann, and many others.

The literature of medical mycology since 1900 includes many thousand titles. No attempt will be made in this paper to give a complete bibliography, even for recent years. Many of the papers are short case reports which increase our knowledge of the geographical distribution, the prevalence, or the clinical variations of a disease. These titles can be readily found in some of the indexing and abstracting journals.

It will be most convenient to take up the mycoses individually and note some of the significant contributions to each. Besides the more common fungous diseases which will be discussed here, there are many isolated cases of infection believed by the reporters to be due to fungi not ordinarily considered pathogenic. Our knowledge of these infections must remain incomplete until additional cases are studied and reported.

Many of the papers in the literature of medical mycology deal in part with taxonomic controversies. Some of the changes proposed are the logical result of new information gained about a given fungus or the disease it produces. Others are due to the difficulties of interpreting early studies and descriptions of fungi.

ACTINOMYCOSIS

Many recent papers on actinomycosis are concerned with nomenclature. This is due in part to a disagreement among investigators about the correct name of the most important species. The etiologic agent of actinomycosis was named *Actinomyces bovis* by Bollinger

and Harz (34) long before it was obtained in culture. Subsequent attempts to culture the organism yielded to Wolff and Israel (308) a microaerophilic species of *Actinomyces*, and to Bostroem (36) an aerobic species. A microaerophilic species is the etiologic agent of the common type of actinomycosis in both man and cattle, and there seems to be little doubt that this is the organism to which Harz applied the name *A. bovis* (62, 198, 108, 249). Negroni (246, 249) prefers the name *A. israeli* for this microaerophilic strain. In view of the circumstances under which Bostroem isolated his species, and the experiences of later investigators, it is probable that his aerobic form was a contaminant. However, some investigators have reached different conclusions and reject the name *A. bovis* (13, 14, 15, 258).

Confusion regarding the etiology of the disease and use of the name *A. bovis* has resulted in what is probably a misconception regarding the habitat of the fungus in nature, and the source of infection. Those who accept the aerobic species of *Actinomyces* as the etiologic agent of "lumpy jaw" and related forms of actinomycosis are inclined to accept the traditional explanation of the introduction of the fungus into the tissues of the buccal cavity on such objects as straws and the awns of grasses. Aerobic species of *Actinomyces* similar to Bostroem's fungus are common in soil and decaying vegetation. The microaerophilic species, however, has not been isolated from these substrates, but it is known to be commonly present in and about carious teeth, in dental scum, and in the crypts of the tonsils (196, 197, 198, 199, 236, 238, 31, 109, 110, 71). Several investigators (237, 278) have stated that microaerophilic species of *Actinomyces* play a role in tartar formation on the teeth.

It is a common observation that many cases of cervico-facial actinomycosis are associated with dental caries, and the infection sometimes follows tooth extraction. It is probable that the disease often begins as a mixed infection and that as it progresses most of the bacteria are eliminated (10). Naeslund (238), on the other hand, states that the association of bacteria is not a necessary prerequisite to infection, but that the presence of bacteria may hasten progress of the infection to contiguous tissues.

Actinomycosis in cattle must be differentiated from actinobacillosis described by Lignieres and Spitz (192, 296, 290, 275). The two diseases have similar but distinguishable clinical characteristics,

and are easily differentiated by examining a Gram stained smear to determine whether the Gram positive diphtheroid elements of actinomycosis or the Gram negative rods of actinobacillosis are present.

Actinomyces bovis in man falls into three main clinical categories, viz., cervico-facial, comprising about 60 per cent of cases; thoracic, 14 per cent; and abdominal, 8-18 per cent (134, 272, 246). Other clinical types are rare. The cervico-facial type is often associated with dental defects or accidents, and is a chronic infection which often remains localized and can be successfully treated. In the thoracic and abdominal types the prognosis is much more grave. The primary lesion of abdominal actinomycosis is apparently often in the appendix (67, 299, 213). In all cases there are usually draining sinuses, and at autopsy abscesses can often be found in the liver (60). Meningitis (231) and endocarditis (295) are occasional manifestations of the disease. Generalized actinomycosis with hematogenous spread is occasionally seen (43). It is usual to find in the pus from actinomycosis "sulphur granules" consisting of masses of radiating hyphae which terminate in "clubs," but these characteristic structures may be lacking in those cases in which there has been a rapid spread of the infection, especially in actinomycotic meningitis and empyema, and even in occasional cases of the drainage from sinuses (108, 212, 246).

Sanford (271, 272) analyzed 678 cases of actinomycosis in the United States, 209 of them reported in the American literature. The cases he was able to report were for the most part from the upper Mississippi valley, New York, Massachusetts, and Maryland. He pointed out, however, that this did not indicate the actual distribution of the disease, but rather the regions in which it was recognized. His report has been cited, probably improperly, to bolster the theory that the habit of chewing straws leads to infection by *Actinomyces*.

Treatment has been rather unsatisfactory, and has consisted for the most part of surgical drainage, X-ray, and the use of iodides (91). The beneficial effects of radiation are not due to its fungicidal effect, but to its effect upon the host (9). Beneficial effects from the use of thymol (235, 16, 115), vaccines (61, 246, 181, 182), and sulphanilamide (297) have recently been reported.

Besides *Actinomyces bovis*, several other species of *Actinomyces* are pathogenic, particularly the acid-fast species such as *A. aster-*

oides which frequently causes brain lesions (147, 132, 135). The possible relationship between these acid-fast species of *Actinomyces* and the organisms of tuberculosis and leprosy has been pointed out (166, 167).

The serologic and allergic reactions elicited by species of *Actinomyces* have received considerable attention. There is considerable evidence that infection by *Actinomyces* is often dependent upon a previous sensitization of the host (214, 136).

MONILIASIS

Moniliasis is a disease of very diverse clinical types, caused by some species of the genus *Monilia* Bonorden 1851, a group of yeast-like fungi quite different from those included in *Monilia* Persoon 1797 (22). The generic name of *Monilia* is widely applied to the former group by medical mycologists, although it is generally recognized that it is a misnomer. A large part of the current literature on *Monilia* is a controversial discussion of the systematic position, nomenclature, and identification of species of these fungi. Some of the errors in attempted revisions of the group, and the erection of many new species names within it, are largely responsible for its confused status.

Dodge (99) subdivided the genus, reviving a number of old generic names. Synonyms of *M. albicans*, the common pathogenic species, appear in several of these subdivisions (179). Diddens and Lodder (97a), in a recent paper, urge acceptance of the name *Candida* Berkhout, a name which is coming into wider use, in place of *Monilia*. Ciferri and Redaelli (59a), on the other hand, prefer *Mycotorula* Will. The generic name, *Candida*, is probably preferable, and will be used in this review.

Castellani has named a large number of species, based largely on fermentation reactions. He has recently (49) reviewed and slightly revised his classification. Baeza (12), working in Castellani's laboratory, has reaffirmed the specificity and permanence of the fermentation reactions. Most investigators, however, do not recognize the validity of some of Castellani's species. The specificity of the fermentation reaction depends upon the purity of the strain (which may be mixed with either another species of *Candida* or with bacteria), the purity of the sugar, and the amount of inoculum used. Methods of identifying species of *Candida* have been

recently outlined (175, 286, 150, 151, 209, 161, 69, 28, 179). Martin (209) and his associates outline an elaborate and carefully controlled procedure which seeks to avoid the sources of error in some of the previously described methods, and takes into consideration the growth habit on broth, Sabouraud's agar, and blood agar, the morphology on cornmeal agar, and the fermentation reactions of the carefully purified strain while under vaseline seal. Langeron and Guerra (179) reject the classification formerly endorsed by Langeron and his associates, make a critical review of the literature, describe in detail methods used in identification, and recognize seven species or groups in the genus *Candida*. One factor contributing to the difficulty in identification of species is the inherent variability of these fungi. Rough and smooth forms are recognized (243, 173, 203, 50). These forms are reversible and both strains may be isolated from the same individual. The range of variation is greater, however, than is indicated by a simple choice between rough and smooth.

The most important species is *C. albicans* which is an etiologic agent in moniliasis of the skin and nails (152, 187), and in thrush and mycotic vaginitis (240, 241, 55, 32, 125, 309, 298). Many investigators believe that it is also of etiologic importance in pulmonary moniliasis (277, 311, 157, 80, 118, 168) and perlèche (117, 268, 122). However, Sebrell and Butler (Pub. Health Rep. 53: 2282-2284, 1938) presented evidence that a riboflavin deficiency is an important factor in perlèche. They did not investigate the possible role of *Candida* in their cases. It has been recently claimed also that *C. albicans* is the cause of "blackhead" in turkeys (301).

The etiologic role of *C. albicans* in thrush, paronychia, and certain types of skin lesions can hardly be disputed. Its role in pulmonary disease is less certain. Shrewsbury (277) states that pulmonary moniliasis and bronchomoniliasis, as postulated by Castellani, do not exist in England. The fungus is commonly present in sputum, and secondary infections of the bronchi occur, whatever primary infection may be present. Ying (311) found secondary infection by yeast-like fungi in nine of 100 cases of pulmonary tuberculosis, and in one of 20 normals. This difference was not observed by Keiper (168) who found *C. albicans* in 3 per cent of normal individuals and 2.9 per cent of those with pulmonary disease. *C.*

albicans apparently is not commonly present on the normal skin (24, 70), but it is often found in the normal mouth. Todd (292) found it in the mouth or throat of 14 per cent of 1,000 normal individuals. Knighton (172) found this species in 23.9 per cent of 146 oral cavities he examined, usually in the throat or on the tongue. None of these individuals had thrush or perlèche. He found no correlation between the presence of *C. albicans* and dental caries or diseases of the gum. The differences in these reports of the incidence of *C. albicans* in the mouth and in sputum are probably due to different methods used in making cultures. So far none of the studies has offered a practical solution to the problem of properly evaluating occurrence of this pathogenic, yet often harmless, fungus in sputum from undiagnosed pulmonary disease.

Specific polysaccharides can be isolated from *C. albicans* and other yeast-like fungi (170). Negroni (242, 245, 247) believes that this material comes from the capsule of the cell.

Candida infection usually responds readily, except in severe and generalized cases, to therapy with potassium iodide, gentian violet, potassium permanganate, alkaline washes, or chrysarobin.

TORULOSIS

An increasing number of case reports and short papers attest the importance of infections caused by the fungus usually known as *Torula histolytica* or *Cryptococcus hominis*. The literature concerning these fungi is confusing. Benham (23) has pointed out that the disease known in this country as torulosis is similar to and has the same etiology as "European blastomycosis." The best known clinical manifestation in the United States involves the central nervous system, while ulcerative lesions of the skin and underlying tissues seem to be more common in Europe. Freeman (124), in an important paper, reported a thorough study of several cases in 1931. Many short case reports have appeared in recent years. A study of the life cycle of the fungus made by Todd and Hermann (291) revealed a hitherto unknown type of sporulation which places the fungus in the ascomycetous genus *Debaryomyces*. They refer to the fungus as *D. hominis*. On grounds of priority the name probably should be *D. neoformans* (Sanfelice) Red., Cif., & Gio. 1937 (263).

SEBORRHEIC DERMATITIS

It has long been known that a small yeast-like organism (*Pityrosporum ovale*) is almost constantly present in the squames of seborrhea. Several investigators have attempted to isolate this organism in culture but have been unable to maintain subcultures. Ota and Huang (254) isolated from seborrhea a yeast-like organism, probably *P. ovale*, which grew very poorly on most culture media. They concluded the organism is a harmless saprophyte. Moore (255) isolated a yeast-like organism which grows readily on the usual media. Despite the fact it differed from strains previously isolated, he believed it was *P. ovale*. It was claimed that *P. ovale*, as represented by this strain, is the etiologic agent of seborrheic dermatitis (229, 171). MacKee and his associates (201), however, found *P. ovale* in 70 per cent of normal scalps and in 80 per cent of all scalps in one series; in 52 per cent of the scalps in a second series (202). Benham (23a) has successfully isolated and subcultured a delicate yeast requiring the addition of lanolin or other fatty material to the culture medium. It appears to be *P. ovale*.

DERMATOPHYTOSIS

The dermatophytoses, popularly known as ringworm, tinea, dhobie itch, athlete's foot, Honkong foot, *etc.*, are the commonest of all fungous diseases. They are more or less superficial infections of the skin, hair, and nails, and the severity in a particular case depends upon the species of fungus involved, the location of the lesion, the presence of a secondary bacterial infection, and the hygienic habits, the susceptibility and often the age of the patient. Strictly speaking, dermatophytosis is a disease caused by any one of the fungi known as dermatophytes. However, there are several conditions which are caused by unrelated fungi, but which are usually thought of in connection with dermatophytosis. There are also a number of reports of unusual skin eruptions in which fungi not usually recognized as pathogens have been isolated. It is possible that in some of these cases the isolated fungus was a chance contaminant, represented on the skin not even by saprophytic growth, but only by air-borne spores which grew out on the culture medium when material was planted. It is probably true, however, that some of the cases will be confirmed by the finding of additional

infections caused by fungi which become pathogenic under special circumstances or only in susceptible individuals.

The dermatophytes have certain morphological features in common and are physiologically specialized for growth upon the keratinized tissues of man, animals, and birds. Recent papers (130, 250, 140) discuss the metabolic activities of these fungi. They appear to be related to each other, and their systematic position is generally believed to be among the lower Ascomycetes, although the actual production of asci has not been proved.

The literature to 1933 dealing with the dermatophytes has been well summarized by Gregory (139). Most of the papers reviewed here appeared after that date.

Three problems are of paramount importance in studies of the dermatophytes: the diagnosis and treatment of the disease, and the identification of species of fungi. The diagnosis of dermatophytosis can not be made with certainty in all cases on clinical grounds alone. Laboratory evidence of the presence of a dermatophyte by microscopic examination or culture is essential in any scientific study of dermatophytosis, and is desirable in all cases presented for treatment. While the experienced clinician is usually correct in his diagnosis, there can be little doubt that many cases of chemical dermatitis and hypersensitivity are incorrectly diagnosed as dermatophytosis and fail to respond properly to treatment with fungicides. In particular, vesicular and scaling lesions of the hands are rarely due to the presence of fungi in these lesions. They may be "dermatophytids" (*i.e.*, an allergic reaction to toxins liberated by a dermatophyte from some focus of infection such as the feet); they may be caused by some other allergen; or they may be due to some chemical irritant. It is said that in some cases a previous fungous infection may sensitize the patient to an allergen to which he was not formerly hypersensitive (302). It is also claimed that a dermatitis may increase the patient's susceptibility to dermatophytosis (164).

For the laboratory examination of material the roofs of vesicles are clipped off with sterile scissors, or the skin at the active edge of a lesion is stripped back. A part of this material is planted on agar and part is mounted in 10 per cent sodium hydroxide and examined with the microscope. Although the dermatophytes produce a characteristic sporulating mycelium in culture, they are

present in the skin and hair only as continuous or fragmented hyphae. These hyphae, if present, become visible as the specimen clears. Lactophenol is also used as a clearing agent, and the specimen is said to be still suitable for planting after examination in lactophenol (45). An artifact which may cause confusion in the microscopic examination is the so-called "mosaic fungus." The probable nature of this material has been discussed by several investigators (287, 101). Davidson and Gregory (79) state that it is a deposition of cholesterol crystals. In most cases it bears no relationship to fungi, alive or dead.

Other methods of demonstrating the fungi have been described. The dermatophytes will grow out from infected scales and hairs if the latter are placed in moist chambers (75, 76). Suspected material can be incubated in this way for diagnosis instead of being planted on agar (33). A diagnosis can not be based on this examination, however, unless the characteristic dermatophyte spores are seen, because spores of some saprophytic fungi which may be present on the specimen can also grow under these conditions. Several papers (287, 5, 26) describe methods of staining material to facilitate the demonstration of fungi. The fact that hairs infected by dermatophytes fluoresce under filtered ultraviolet radiation, as pointed out by Margarot and Deveze in 1925 is frequently used as an aid in diagnosis and a test for clinical cure (77, 78, 184). Different species of dermatophytes vary in the character and amount of fluorescent material and this material can be extracted from infected hairs and fungi (74, 185). Inexpensive units for production of filtered ultraviolet have been described (73, 185).

There are many conflicting reports on the value of fungous extracts in the diagnosis of dermatophytosis. Wise and Wolf (306) give a good discussion of the background of skin testing, and point out the precautions which must be observed in interpreting the reactions which follow intradermal injections of extracts of fungi. Lewis, MacKee and Hopper (188), reporting experimental work and reviewing the status of skin testing with trichophytin, conclude that the reaction is specific as to the genus of dermatophyte, and that in spite of occasional paradoxical results, if the intracutaneous test is carefully made it is a useful adjunct to other methods of establishing a diagnosis. DeLamater and Benham (88), in careful studies of the primary experimental lesions in guinea pigs and of

immunity and hypersensitivity produced, found that in experimental animals the clinical lesion varies with the strain of fungus, and, in the case of a given strain, with different animals. Sometimes indistinguishable lesions were produced by widely different species of dermatophytes. The clinical course of the disease is shortened either by a strong reaction on the part of the host to the parasite, or by a lack of virulence in the parasite. The primary infection alters the animal's reaction to a subsequent infection, as others have previously showed. The second infection may resemble the first clinically, but it is usually sterile and heals quickly. Catanei (54) studied the blood changes in infected, immune and allergic guinea pigs. Henrici (148) found that guinea pigs which had recovered from infection with *T. mentagrophytes* reacted to intraperitoneal injections of live spores, cell sap, or polysaccharide by generalized erythema and desquamation. He considers this condition identical with trichophytid in man, and his experiments strongly support the theory that trichophytid is an allergic response of the skin to substances in solution distributed to it by the blood.

Treatment of dermatophytosis is based ordinarily on the use of a fungicide combined usually with a keratolytic agent which will increase penetration of the medicament and induce peeling of the infected epidermal layers. In ringworm of the scalp it is usually necessary to epilate the patient. A notable exception is in the case of infection caused by *Microsporum canis* (189, 186) which has long been recognized as more responsive to treatment than infections due to *M. audouini*. Infections of the scalp caused by many dermatophytes are more resistant to treatment because the fungus penetrates to the hair root without, however, exciting sufficient reaction to cause its expulsion. Thallium acetate has been and still is used to induce fall of hair (78, 191). X-ray radiation is usually considered a safer method, but here, too, the dosage must be carefully measured (176).

Various fungicidal ointments have been used in treating dermatophytosis (306). The choice depends largely upon the clinical condition of the lesion and the response to therapy. It is often advantageous to alternate remedies. Maynard (215) recommends the use of tri-ethanolamine in the preparation of a number of prescriptions, because of its tendency to lower surface tension and increase the tolerance of the skin for fungicides. Iodine and sodium

hypochlorite are strongly fungicidal when tested in the laboratory (106). Dihydroxy-anthranol (220), sec-amyltr cresol and *o*-hydroxyphenylmercuric chloride (104), sodium borate (100), thymol and a number of the dyes (205), para-nitrophenol (155), and iodocholeate (183) have been recently recommended. Ultraviolet radiation, particularly in the wavelength range 2537–2650 Å, is fungicidal for spores of *Trichophyton* (111, 149). For laboratory testing, blocks of agar and mycelium from an inoculated plate (39) or spores can be exposed to the fungicide. Several recent papers advocate the local use of citric acid, vinegar, or lemon juice (20, 3) in therapy of dermatophytosis. Sweat is itself fungicidal *in vitro* due probably to its acid content (255). Attempts to introduce metallic salts by electrophoresis into the skin have not been uniformly successful, although this method is sometimes advantageous in dermatitis cases (200). The general aspects of electrophoretic therapy are discussed by Harpuder (143).

There have been several enthusiastic reports of the use of vaccines and extracts of fungi in the treatment of dermatophytosis. The favorable results reported by Robinson and Grauer (267) are open to question because it appears from their paper that their diagnosis was based in part on the isolation in culture of such fungi as *Aspergillus* and *Penicillium* from some of their cases. There seems to be a definite place for treatment of selected cases (patients who are sensitized to the dermatophytes) by desensitization with vaccines (294, 305, 188, 293), but the method is not to be depended on routinely. Whatever type of therapy is used in the treatment of such conditions as dermatophytosis of the foot, it is very important that it be supported by proper hygienic measures such as keeping the foot clean and dry (138, 123).

Prophylaxis has been directed toward attempts to kill the fungi present in desquamated infected skin, or to prevent dissemination of the fungi from this source. A foot-bath containing 1 per cent sodium hypochlorite has been recommended (253) for use in locker rooms. Shoes worn by an infected individual may contain dermatophytes (160). Fumigation of shoes by placing them in a box with formaldehyde aids in preventing recurrence in the patient after cure (11, 146). Bonar and Dreyer (35) reported that while dermatophytes did not grow on clean sound wood, they did grow on wood covered with a film of slime. They found that the time re-

quired to kill *Trichophyton interdigitale* in naturally infected skin squames placed in 1 per cent sodium hypochlorite solution was one hour or more. It was killed by ten minutes exposure to a temperature of 75° C. Standard power laundry practice can be depended upon to kill the fungus in white fabrics, but the processes to which silk and woollen fabrics are exposed probably are not effective. Standard "dry cleaning" solvents are not appreciably fungicidal. Berberian (27) found the fungus capable of growing on stockings and reported that it can survive home laundering.

The ability of species of *Trichophyton* to grow on cereal grains, litter, and debris of various kinds is well known. Their natural occurrence as saprophytes was reported by Muende and Webb (233) who found *T. roseum* and *T. gypseum-asteroides* growing on dung in a shed occupied by infected calves.

The incidence of dermatophytosis of the foot is high. Legge, Bonar and Templeton (180) found that 51.5 per cent of the men admitted in one year to a university had dermatophytosis of the foot, and that the incidence increased to 78.6 per cent by the end of the year. Alderson and Reich (4) found that this disease represented 24.7 per cent of the skin diseases presented in a student health service. Williams (303) has analyzed for age, occupation, etc., the data recorded in 2400 clinical histories of dermatophytosis in the Boston City Hospital. Lomholt (195) reports an incidence of 33-50 per cent in school children in Copenhagen. In most of the cases studied in these surveys a clinical diagnosis only was made. Downing, Nye, and Cousins (103) obtained cultures of two dermatophytes from apparently normal skin between the toes. It is generally accepted that the clinical diagnosis can not always be depended upon, because of subclinical lesions or of dermatitis resembling dermatophytosis. The incidence is high, however, when determined by either clinical or laboratory diagnosis. A laboratory diagnosis of dermatophytosis of the foot was made by positive microscopic examination or culture, or both in 36.4 per cent of 354 men in an industrial plant (239).

The most workable classification of the dermatophytes, and the one in most general use, is that of Sabouraud (269). He reviewed the basis upon which his system rests in 1929 (270). Useful as this classification has been, it has certain imperfections, and several so-called "botanical classifications" have been proposed to replace

it. These have not clarified the problem because the points most fundamental and important for a natural grouping were ignored, and dermatophytes which are obviously closely related were widely separated. Gregory (139) reviews these attempts at systematic study and they will not be discussed further here.

The writer believes that the groups Sabouraud proposed are, with some exceptions, natural groups, and placing the emphasis on the mycological characteristics instead of the clinical aspects makes this more apparent. One difficulty is presented by the genus *Achorion* which can be defined only as a group including those fungi which cause favus. This is not a proper definition for a genus, and attempts to retain the name result in a duplication of names. One species is the usual etiologic agent in favus, but some species of *Microsporum* and *Trichophyton* occasionally cause favus. In the writer's opinion (107), the genus should, therefore, be dropped and the species distributed to the genera in which their obvious affinities lie.

The question of synonymy of species is important. Some 200 species names of dermatophytes are in the literature. Some of the earlier evidence that many of these are synonyms is reviewed and other evidence presented in the paper by Emmons (107) already referred to. The commonest etiologic agent of dermatophytosis of the foot over much of the United States is a fungus called by several names, best known of which are *Trichophyton gypsum*, *T. interdigitale*, and *T. pedis*. Evidence that these represent merely varieties of one species is given in various papers. Dowding and Orr (102) isolated *T. gypsum* from three clinical types of ringworm, tinea circinata, scaly or vesicular lesions of the feet, and deep inflammatory lesions (kerion). The strains of fungi differed considerably from each other in color and surface texture, but there was no positive correlation between the clinical type of lesion and the variation in growth of the fungus on agar. Epstein (114) concluded from studies based on variability, animal inoculations, and serological and clinical investigations that *T. interdigitale* is a degenerated form of *T. gypsum*. Neal and Emmons (239) reported studies of 71 strains of this fungus isolated within a period of one month from the personnel of a large printing establishment. These strains formed an intergrading series between the more granular varieties of the *T. gypsum* type and the more floccose varieties of

the *T. interdigitale* type of growth. In view of the variability of these forms, and the confusion resulting from the use of several different names there is much to be said in favor of referring them all to the species name *T. mentagrophytes* Robin 1853 (107). Without doubt the dermatophytes do cross what are considered by some medical mycologists to be species lines, and the variations or mutations may occur spontaneously in old cultures (105), but the frequency of their appearance can be greatly accelerated by exposure to such extraneous influences as ultraviolet radiation (149, 111). The rate at which mutations are induced by monochromatic ultraviolet radiation reaches a maximum in the wavelength range 2537–2650 Å. Within this range, the percentage of surviving spores which give rise to mutants is greatest when 50 per cent to 99 per cent of the spores are killed. With increasing energy the percentage decreases rapidly.

Conant (64) attempted by biometric studies to determine which of the described species of *Microsporum* were valid. He measured a large number of spores, but he did not base his studies on a large number of strains of the species investigated. An examination of additional strains of *M. fulvum* and *M. gypseum*, for example, probably would have showed that one is a synonym of the other. Some new species names which have been published are evidently given because the describers do not admit that variation or mutation can take place during the parasitic phase as well as in the saprophytic phase of growth (178, 21).

Probably dermatophytosis of the foot is as prevalent in temperate climates as in the tropics, but other types of dermatophytosis are seen more often in warm climates. This is probably due in part to the geographical distribution of species, to the greater tendency toward extension over the general body surface (which depends also in part on the species of dermatophytes), and to the poor living conditions of masses of the population in some warm countries.

An attempt to determine the geographical distribution of species is made more difficult in some cases by disagreement about the correct names and taxonomic limits of species of dermatophytes. Certain generalizations can be made, however. Dermatophytosis of the foot in north and central United States may be caused by *Epidermophyton floccosum*, *Trichophyton purpureum*, or *T. mentagrophytes*, but is usually caused by the latter. In southern United States,

in Puerto Rico (40), and in many warm countries *T. purpureum* is more common. This species tends to become generalized over the body surface in the tropics, and may do so in temperate climates (190). The species involved in dermatophytosis of the body may be determined by the patient's occupational exposure to cattle, horses, dogs, or other animals. Catanei (52, 53), who has made extensive studies in Algeria and neighboring countries, reports that next to favus, tinea caused by endothrix species of Trichophyton, especially *T. violaceum*, is most prevalent. This is generally true in south Europe, northern Africa (177, 131), India (92), and China (where *T. purpureum* is also common) (251, 232). Favus is rare in the United States, and when found is often reported. Many of the cases are in immigrants or children of immigrants, but Barrett (17) reports what appears to be an endemic focus in Kentucky.

BLASTOMYCOSIS

American blastomycosis (Gilchrist's disease), is caused by *Blastomyces dermatitidis*. The disease was described in detail by Gilchrist in 1896 (127) but the etiologic agent was not isolated at that time. In a second case Gilchrist and Stokes (128) isolated the causative fungus which they tentatively classified as a species of *Oidium* and later (129) named *B. dermatitidis*.

The name blastomycosis has been used very loosely. The principal confusion is with "European blastomycosis" which was discussed under the name Torulosis. Redaelli (259) lists nine different diseases as types of blastomycosis, and advances reasons for abolishing the name. Few authors would use the name in such a broad sense, but it has been used to designate diverse conditions in which budding cells are found in the parasitized tissues. Various papers (221, 145, 283, 48, 23, 259, 260, 227) have pointed out the differential characteristics of the diseases most commonly confused, and of the fungi causing them.

It is generally recognized that, strictly speaking, *Blastomyces* is not a correct generic name for the fungus. The name has been frequently changed. Martin and Smith (211) state that 19 new names (including synonyms and generic changes) have been published, and that they prefer the name *Blastomyces*. Castellani (48) proposed to substitute *Blastomycoides*, Redaelli and Cifferi (260)

proposed *Gilchristia*, and Dodge (99) uses the name *Zymonema*. Both *Blastomycoides* and *Zymonema* contain unrelated fungi.

The fungus is found in the lesions in giant cells, or intracellularly in pus or sputum, as rather large (8–10 μ in diameter) thick-walled budding cells. When these cells from the parasitic phase are planted on agar they produce a mycelium which is dirty white or brownish. Conidia are borne on short lateral conidiophores on this mycelium. When an animal is inoculated with spores or hyphae the fungus reverts to the budding phase. The budding type of growth can be maintained in culture if conidia or the parasitic cells in pus are planted on blood agar (90, 206). Most investigators have not confirmed the claims of Moore (223a) and Dodge (99) that *Blastomyces dermatitidis* produces asci.

DeMonbreun (90) reported a case of chronic infection with *Blastomyces* and the successful experimental production of chronic cutaneous blastomycosis in the monkey. The paper is critically written and well illustrated. Martin (206) and Martin and Smith (210) outline the usual direct methods of laboratory diagnosis of the disease, and describe a skin test and a complement fixation test which are useful in establishing a diagnosis, particularly in those cases where skin lesions or a productive cough are lacking. In a later valuable and well written paper Martin and Smith (211) analyze 347 cases of reported blastomycosis. They consider only 80 of these to be proved as caused by *B. dermatitidis*, and 163 cases were classed as presumptive. Thirteen of the cases were personally studied by the authors, and case histories of these are given. The cases are analyzed as to age, sex, race, and geographic distribution, organs involved, and the allergic state. The authors state that the mortality rate is much higher than in tuberculosis. Treatment with iodides, while often successful in patients with the cutaneous type of infection, is not effective in the systemic disease, and its administration is dangerous in patients who are allergic to the fungus. They recommend that complete or partial desensitization of allergic patients be attempted by the use of a heat-killed vaccine before other treatment is attempted.

COCCIDIOIDOMYCOSIS

Coccidioidomycosis (94) or coccidioidal granuloma, first reported in South America (300) and later in the United States

(264) is a generalized disease caused by the fungus *Coccidioides immitis* Stiles 1896. The fungus exhibits marked dimorphism. It grows on agar as a white mold in which sporulation occurs by the fragmentation of specialized hyphae into chlamydospore-like oidia. When these spores are injected into an animal they become spherical, increase in size, and finally reach a diameter of 50–70 μ . Each of the large spherical cells then functions as a sporangium, and the cytoplasm gives rise by cleavage to large numbers of spores which fill the sporangium. These escape by rupture of the sporangial wall and repeat the parasitic phase of the life cycle. Dickson (95) reports a critical study of the processes of growth and sporulation of the fungus and summarizes earlier studies on the subject. Moore (223) and Dodge (99) refer to the sporangia as asci, and Ciferri and Redaelli (56, 57) report conjugation in *C. immitis*, but these reports lack confirmation. Sporangia and endosporulation can be induced *in vitro* by incubating in Locke's solution and serum (56, 57) or in the presence of partially coagulated egg albumen and under partial anaerobiosis (174).

The earlier history of the disease is well summarized in a bulletin of the California Department of Public Health (265). From the time when the first case was published (1892) to 1931, when the bulletin was prepared, 286 cases had been reported, 89.5 per cent of them originating in California. By July 1, 1936, 450 cases with 224 deaths had been reported in California alone (8). Of these, 85 per cent were males and 61 per cent were between the ages of 25 and 55. Most of these patients were exposed in varying degrees to soil or plants, and it is believed that the fungus grows in soil or on vegetation. Stewart and Meyer (284) isolated it from soil in a feed lot heavily contaminated by infected cattle, but its natural habitat is not known.

While the disease is more common in California than elsewhere an increasing number of cases have been reported from Texas (280), Arizona (257), South America (87), and isolated cases from other parts of the world.

Since publication of the Bulletin knowledge of the disease has been extended by the important studies of Dickson who had pointed out that infection by *Coccidioides* was probably much more common than had been recognized, and that the apparent mortality rate was probably much too high (93). Later he (94, 96, 97) and others

(279, 126) showed that "Valley fever" is a manifestation of primary pulmonary coccidioidomycosis from which the patient usually recovers spontaneously and without apparent residual infection. It has been shown also that a high percentage of residents of the San Joaquin Valley react in such a way to skin tests with "Coccidioidin" as to indicate that they have been intimately exposed to *Coccidioides* (156, 169) and the reaction is especially severe in individuals who have had "Valley fever." Kessell (169) considers the coccidioidin skin test specific for coccidioidomycosis.

Further evidence for the previously unsuspected prevalence of the disease in endemic areas was supplied by the finding at necropsy of patients dying of other diseases, healed lesions of coccidioid granuloma (93). Four such cases were reported by Cox and Smith (68) who were able also to produce arrested lesions in white rats. The fungus was isolated in culture from one of their patients with a chronic skin lesion 15 years after the first isolation, and could be recovered from the arrested lesions in their experimental animals after two and one-half years. Such lesions are, therefore, to be regarded as potential sites for reactivation of the infection.

Treatment of coccidioidomycosis is not satisfactory. Before the occurrence of more benign forms of the disease were recognized it was believed that early surgical excision and amputation were the only effective treatments. It is now known that there is spontaneous recovery in some cases and that some patients respond to treatment. Jacobson (158, 159) recommends the use of colloidal copper and vaccines. Sox and Dickson (281) tested a number of drugs in experimental infection in guinea pigs and found only thymol to be of value in increasing length of life of the infected animals. Many clinicians now agree that a strict regimen of bed-rest is the best basis for treatment (116).

Beck (18), Beck, Traum and Harrington (19), and Davis, Stiles, and McGregor (81) have reported the disease in cattle. There is little evidence, however, of transmission of the disease from animal to animal, animal to man, or man to man. Probably man and animals are infected from a common source.

The disease is protean in its clinical manifestations. The primary pulmonary type of infection has been mentioned. Probably even in those patients who do not show the acute type of infection known as "Valley fever," infection occurs, in the majority of cases,

by the pulmonary route. Primary skin lesions have also been reported. Carter (46, 47) discusses infectious granulomas of the bones and joints and the pulmonary infection as revealed by X-ray. Cavity formation in the lung, while not the rule in pulmonary mycoses, has been reported (116). Coccidioidal meningitis is a not uncommon manifestation (1).

PARACOCIDIIDIAL GRANULOMA

Paracoccidioidal granuloma which has been confused with coccidioidomycosis is caused by a different fungus, *Paracoccidioides brasiliensis* (Splendore) de Almeida, and has different clinical characteristics. DeAlmeida (87) pointed out these differences, and Jordan and Weidman (162) confirmed this differentiation and proposed that the disease be called "Almeida's disease." The fungus does not cause a generalized infection in experimental animals and in man infection usually occurs by mouth and may be limited to the buccal mucosa and adjacent skin surfaces. The gastrointestinal tract is usually involved. The fungus itself differs from *C. immitis* by more restricted growth and sporulation on agar, and by reproduction by budding instead of by endosporulation in infected tissues. Moore (226) described a new species, but other investigators (248, 261) reject this separation. Moore (227) has since proposed a second new species. Redaelli and Ciferri (261) have made an extensive report of their studies of the fungus, and conclude that its systematic position is among the Chytridiales. Negroni and his associates (248) present a good discussion of the fungus and the clinical aspects of the disease.

SPOROTRICHOSIS

Sporotrichosis was first described by Schenck in the United States in 1898. The causative fungus, *Sporotrichum schencki*, was named by Hektoen and Perkins (144). The disease was later reported from France by Beurmann and Ramond (30) who believed they had a different species of the fungus, *S. beurmanni*. The fungus also causes a disease of horses, and has been called *S. equi*. It is a common observation that the type of growth and degree of pigmentation of a given strain varies with conditions in the culture and the interval since isolation. Critical studies have indicated that the three names mentioned, and probably several others, are syno-

nymy (82, 83, 84, 85, 86, 25). Greenburg (137) reports a case in which the fungus isolated from the original chancre resembled *S. schencki*, that from a nodule resembled *S. beurmanni*. *S. gougeroti*, *S. councilmani* (141), and perhaps a few other species are probably distinct, but the pathogenic fungi which have been placed in this genus need critical review.

Beurmann and Gougerot (29) have discussed the group at length, and have classified the various clinical forms. This classification has been accepted by other investigators (119, 153). In the most common form of the disease seen in the United States a primary lesion, often on the finger, appears at the site of some minor injury such as a thorn prick. It ulcerates, fails to heal, and is followed by the appearance of subcutaneous abscesses along the course of the regional lymphatics. In France a disseminated form is more common in which many subcutaneous nodules appear, probably as the result of hematogenous spread. This type is occasionally seen in the United States (72). The other types, involving principally the epidermis, the mucous membranes, the skeleton, and the viscera, are less common. Forbus (121) has discussed pulmonary sporotrichosis. He reports a case and reviews nine other published cases. He believes that only one of these was actually pulmonary sporotrichosis. From a careful study of his report it would appear that this one case could hardly have been caused by *S. schencki*. Cortella (66) reports a case of pulmonary involvement, and Wooley (310) isolated the fungus once from sputum.

It is believed that the fungus grows on vegetation and that man and animals are infected from this source. Foerster (119, 120) reviewed published cases of the disease in the United States and reported a series of cases in men who were exposed to injury by barberry thorns. Beurmann and Gougerot isolated what they believed to be the common pathogenic species from various plants. Benham and Kesten (25) cultured the fungus on carnation buds without loss of its virulence. The disease appears to be almost world-wide in distribution. From an analysis of the reported cases it would appear that in the United States the disease is most common in the upper Mississippi valley (119), but it is probable that these reports, as in other mycoses, do not give a true indication of the actual geographical distribution of the disease.

The laboratory diagnosis of the disease is made by culture from a subcutaneous abscess or by injection of pus into an animal prefer-

ably a white rat. Demonstration of the parasitic fungus in the human lesion is difficult. Moore and Davis (222) described an agglutination, a complement fixation, and a skin test; but in most cases the easier and more direct methods are adequate.

The disease usually responds readily to the administration of iodides. Loewe (193) reported a case which was not cured by iodides, but responded favorably to the use of an autogenous vaccine, phenylmercuric nitrate and sodium hypochlorite.

CHROMOBLASTOMYCOSIS

Chromoblastomycosis (*dermatitis verrucosa*) has a very wide geographical distribution (216, 217, 289, 219, 304, 208, 6, 230, 204, 288, 42) although the number of reported cases is comparatively small. The disease is a chronic, localized infection of the skin and subcutaneous tissues manifested as warty or cauliflower-like outgrowths, usually on an extremity. Some of the cases reported have had a duration of as much as 40 years. The infection is most commonly seen on the lower leg, and occurs principally in outdoor laborers, particularly men who work barefoot in the fields. There is no evidence of spread from the patient to other individuals, even those with whom he is intimately associated. This fact, together with the relatively high incidence in barefoot laborers of the peon type in the tropics (some of whom report injuries preceeding appearance of the lesions), and the wide geographical distribution, suggest that this fungus is normally present in soil or decaying vegetation, and that under certain conditions or in susceptible individuals, it will, when introduced subcutaneously on thorns or splinters, become pathogenic. Gomes (133) claims to have isolated a new fungus which caused chromoblastomycosis and which was also "parasitic" on a branch of eucalyptus tree by which the patient was injured. He does not present clear evidence, however, that the fungus isolated was the etiologic agent in the disease, nor that it was the same as the species isolated at a later time from the eucalyptus tree.

The name of the disease, chromoblastomycosis, was given to indicate that the pigmented cells of the fungus bud in the tissues. As a matter of fact, the brown sclerotic cells of the parasitic growth phase of the fungus do not actually bud, but divide by septation. The other common name of the disease, *dermatitis verrucosa*, is not

satisfactory because it includes several unrelated conditions (194).

The disease was first studied by Pedroso in 1911, but this case was not reported until 1920 (256). In the meantime Medlar (216, 217) had reported a case from Boston, and named the fungus *Phialophora verrucosa*. Pedroso and Gomes (256) incorrectly identified their fungus with this species, but in 1922 Brumpt (38) pointed out that it differed and called it *Hormodendrum pedrosoi*. The fungus has obvious close affinities with other species of the *Hormodendrum-Cladosporium* group of fungi, but its reduced type of sporulation has led investigators to transfer it to other genera (289, 38a). The merits and faults of these classifications are discussed in various papers (113, 208). Since the earlier attempts to place this pathogenic fungus in an appropriate genus it has suffered several transfers. Dodge (99) transferred it to *Gomphinaria*; Negroni (244) created the new genus *Fonsecaea*; Briceno-Iragorri (37) proposed *Carrionia*; and Moore and Almeida (228) reported certain differences between strains and split the species, creating three new genera and two new species, as well as one new species of *Phialophora*. Their division of *H. pedrosoi* was based on the type of spore production they observed in the strains they examined, although it had been previously shown (44, 112) that the three important types occur simultaneously in each of a large number of representative strains.

Although more than one species of fungus (*H. pedrosoi*, *H. compactum*, and *P. verrucosa*), is involved, and although the clinical aspects of various cases differ somewhat, there is no reason for separation into two or more diseases. Most of the cases have been due to either *H. pedrosoi* or *P. verrucosa*. *H. compactum* (41) is a closely related species. Cases clinically diagnosed as chromoblastomycosis have been reported in which other fungi were isolated (163, 165). Wilson, Hulsey, and Weidman (304) suggested that the two commonly pathogenic species might be different growth phases of one fungus. Evidence for a very close relationship was furnished by the observations of Carrión and Emmons (44, 112, 113) that the *Phialophora* type of sporulation (*i.e.*, a serial budding of small spores in the base of the cup-like tip of a conidiophore) does occur, although usually rarely, in strains of *H. pedrosoi*. This was confirmed by other investigators (65, 228).

Conant (63) made the very interesting observation that certain fungi which are of economic importance in causing blueing of wood

pulp (218) and which have been described as species of *Cadophora* are actually species of *Phialophora*, and Martin (207) showed by serologic studies that *C. americana* is identical with *P. verrucosa*.

HISTOPLASMOSIS

Between 1906 and 1909 Dr. S. T. Darling, searching for kala azar in Panama, reported his discovery of a disease characterized by splenomegaly, irregular fever, leucopenia, and anemia. He was not able to obtain the etiologic agent in artificial cultures, and because of its appearance he believed it was a protozoan parasite, which he named *Histoplasma capsulatum*. In 1912 Rocha Lima concluded the organism was a fungus related to *Cryptococcus farciminosus*, but he did not secure cultures. During succeeding years a few cases of the disease, widely separated geographically, were diagnosed at autopsy, but no advance was made in the determination of the systematic position of the organism.

In 1934 Dodd and Tompkins (98) diagnosed the condition in an infant, a native of Tennessee, during life and isolated cultures. The pathological aspects of the case and a description of the fungus were published by DeMonbreun (89). In its parasitic phase, the fungus is a small yeastlike organism which invades the mononuclear cells in enormous numbers. In its saprophytic phase on the usual agar media it is a mold which produces conidia, some of which reach a size of 10–25 μ and produce finger-like projections reaching a length of 5 μ . DeMonbreun proposed to change the name of the fungus and of the disease because of Darling's misconception of the systematic position of the organism, but since Darling created a new generic and a new specific name for the parasite, such a change is not necessary.

In the same year Hansmann and Schenken (142) reported a case of this disease in an adult, and obtained cultures of the fungus. They considered the diagnosis of Darling's histoplasmosis, but rejected it because their patient showed no enlargement of the spleen. A comparison of their strain of the fungus with DeMonbreun's, however, shows clearly that the two are alike.

Moore (224) proposed a new species name for one of these strains and transferred both to the genus *Posadasia* because of an alleged resemblance to *Coccidioides*, which he had also transferred to that genus. Reasons for rejecting this separation of the two

strains and this classification are given in a series of papers by Ciferri and Redaelli (59, 262). Howell (154) has compared the fungus with saprophytic fungi to which it bears some resemblance.

Other cases of the infection have been recently reported (234, 274, 2, 7, 276), furnishing further evidence of the wide geographical distribution of the disease and the desirability of considering it in undiagnosed clinical cases of this type.

SUMMARY

In spite of the rather voluminous literature of medical mycology it is the general opinion that the subject is inadequately studied in both the mycology laboratory and the medical school. The fungi causing disease in man have been largely neglected by mycologists. This is due perhaps in part to the dangers incident to handling some pathogenic fungi in the general mycology laboratory, where it is ordinarily not necessary to take any precautions against accidental infection; in part to lack of adequate facilities for using animals in experimental studies; and principally to a lack of contact with clinicians and clinical material from the hospital. None of these reasons, however, justifies ignoring a group of fungi, some of which are fairly common and all of which are important because of their roles in the causation of disease. There are some contributions which can be made from the mycology laboratory with or without the use of experimental animals or close association with the clinic. There are other problems which can best be solved by studies made cooperatively by physician and mycologist.

Physicians, on their side, are less familiar with mycotic diseases than with bacterial diseases. Many of them are disinclined to enter a field which they look upon as a difficult one because of the technics peculiar to it, and because of the enormous number of species of fungi, the classification of which is somewhat chaotic. The systematic arrangement of the fungi of medical importance is unnecessarily chaotic because many describers of new species, not recognizing the importance of variation in fungi, have given new species names where these were not justified; or, failing to examine the descriptions of earlier investigators, have repeatedly renamed species; or have used names already preempted for other fungi. It has been generally supposed, moreover, that the mycoses are relatively infrequent. Whereas, in plant pathology the important etio-

logic microorganisms are fungi, bacterial diseases in plants being comparatively rare, the converse is true in animal pathology. Most of the common diseases of man and animals are caused by bacteria or filterable viruses, and except for such common skin infections as dermatophytosis of the foot (athlete's foot) and some other types of dermatophytosis or ringworm, cases of disease in man caused by fungi are either relatively infrequent or are poorly known. These conditions have probably been responsible for the lack of a development of medical mycology comparable in importance to that of medical bacteriology. Recent studies seem to indicate, however, that the importance of fungi in medicine has been unduly minimized.

The net result of these factors has been that most mycologists have had little or no first-hand knowledge of the various fungi causing disease in man, and relatively few physicians have made notable contributions to our knowledge of the diseases caused by them. Most of the contributions to the subject, both to the taxonomy and nomenclature of medical mycology and to the knowledge of mycotic diseases, have been made by a few physicians who have had a particular interest in the subject. Recently, mycologists have begun to take a more active interest in these fungi.

The fungi of medical importance belong to diverse groups. *Actinomyces* is represented at one end of the line, the type species of that genus, in fact, being *A. bovis*, an anaerobe which causes the common type of actinomycosis or "lumpy jaw." A few Ascomycetes or fungi with obvious ascomycetous affinities are in the group, but most of the pathogens must be placed among the Fungi Imperfecti. The diseases themselves vary as widely as their etiologic agents. They include the most superficial skin infections (such as tinea versicolor) and the more bothersome, yet still superficial skin infections known as dermatophytosis, tinea, or ringworm (including "athlete's foot"); diseases characterized by ulcerations and granulomatous lesions; and diseases which are systemic or generalized. Besides the more commonly recognized mycotic diseases, an infection is occasionally caused by some fungus which is ordinarily unable to invade animal tissue. Even among the commonly recognized mycoses, the evidence indicates that the etiologic agents are normally saprophytes and cause disease in man only when introduced into the tissues under exceptional circumstances, or in a

susceptible individual. There is little or no evidence of spread of infection from one individual to another in most mycoses. An important exception is in the case of dermatophytosis. The pathogenic fungi known as dermatophytes are physiologically adapted for growth on the keratinized tissues (the epidermis, hair, and nails) of man, and readily pass from man to man or animal to man. Some species of dermatophytes are narrowly specific in host requirements. *Microsporum audouinii*, for example, causes ringworm of the scalp in children only, passes readily from child to child, but rarely to adults, and the lesions heal spontaneously at puberty.

Most of the pathogenic fungi exhibit a striking dimorphism. In the parasitic phase the dermatophytes are present in the epidermis, nails and hair as hyphae or hyphal fragments (arthospores). In saprophytic cultures these fungi produce two types of spores and a variety of characteristic accessory structures. Many pathogenic fungi proliferate in the tissues by a budding or pseudobudding process, but when transferred to agar grow with profuse aerial hyphae bearing characteristic spores. In some cases the type of growth found in tissues can be maintained in artificial culture by adding serum or blood to the medium or incubating under partially anaerobic conditions at body temperature. *Coccidioides immitis*, one of the most virulent fungi, produces only large spherical sporangia in tissues, but grows on agar as a widely spreading mold with many spores which break from the hyphae and are readily disseminated by air currents.

Despite the variety of mycotic diseases and of their etiologic agents some generalizations can be made. Henrici (Jour. Bact. 39: 113-138, 1940) has pointed out some of the broad principles which appear to underlie the mycoses, and which differentiate these from the bacterial diseases. He has set forth and reviewed in a clear manner the thesis that the mycoses are characterized by the development of a type of hypersensitivity best designated as the allergy of infection; that certain saprophytic fungi, in other words, are capable of causing mycoses involving the deeper tissues and organs because they are able to produce antigen-like substances peculiar to some fungi and a few bacteria; and that this characteristic distinguishes the fungous diseases from most bacterial diseases, but relates them to a few, such as tuberculosis.

The methods of control of disease practiced by the plant pathologist are obviously not applicable to the control of mycotic disease in man. Wading pans containing a solution of sodium hypochlorite have been used in locker rooms to prevent spread of dermatophytosis of the foot. The treatment of dermatophytosis is usually based upon the use of a fungicide combined with a keratolytic agent which will induce peeling of the infected skin layers. The deeper mycoses are sometimes successfully treated by surgical drainage, X-ray radiation, or medication with potassium iodide, singly or in combination. While potassium iodide is quickly effective in treatment of some mycoses and is considered generally useful in mycotic infections, it is not a specific treatment against this group of diseases. Desensitization of the individual, and improvement of the general condition by rest and proper food are important primary or supporting therapeutic measures in some cases.

By ingestion and inhalation into the upper respiratory passages the individual is frequently exposed to spores of many species of fungi. Occasionally such spores are introduced into the subcutaneous tissues by thorns or splinters. A few fungi live habitually (usually in the role of harmless saprophytes) on the skin and mucous membranes of man. Some of these fungi are capable under exceptional circumstances, as stated above, of becoming pathogens of man. Because of the nature of this exposure, the unpredictable behaviour of the fungi, and the sporadic occurrence of the less common mycoses, it is not surprising that the diagnosis of fungous diseases is difficult, and that the incidence appears to be low. This may be an inaccurate picture of the situation. Recent reports of cases of histoplasmosis, for example, indicate that so far as this mycosis is concerned, it may well be more frequent than the few diagnosed cases indicate. This is probably true also for other mycoses, particularly in geographical areas where the practicing physician is not looking for them, and lacks the laboratory facilities which are important aids in the diagnosis of most fungous diseases.

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THE PHYSIOLOGY OF CELL ELONGATION

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I

INTRODUCTION—DEFINITIONS—THEORIES OF OLDER INVESTIGATORS

The phenomena of growth may be separated into different processes, such as differentiation, elongation and maturation. Of these, only cell elongation will be dealt with in the following pages. The term elongation will be used for any permanent enlargement of cells already formed by differentiation. As the size of a plant cell is determined by the extent of the surface of its wall, cell elongation may be defined also as any permanent increase in the surface of the cell wall.

Although elongation was studied by the earlier botanists, analysis of its mechanism had not yet been made some few years ago, and the views of different authors at that time differed widely. Almost every theoretical possibility was formulated into a theory. The question as to which factor initiates elongation, and which of the many properties and factors, playing parts during or varying with elongation, is the primary and direct cause of elongation and its limiting factor, could not be answered with certainty.

Before considering contemporary ideas on the subject, a short survey of the different views of older investigators will be given, thus providing a clearer introduction to the problem, and some definitions will precede this survey in order to prevent misunderstanding:

Elasticity is the ability to undergo reversible changes in size or shape.

Plasticity is the ability to undergo permanent, irreversible changes in size or shape.

Extensibility is the ability to undergo changes in length.

Elastic extensibility is the ability to undergo reversible changes in length.

Elastic extension is reversible increase in length.

Plastic extensibility is the ability to undergo permanent, irreversible increase in length.

Plastic extension is permanent increase in length.

✓ Increase of cell contents and uptake of water generally occur simultaneously during increase in volume of the cell. In exceptional cases there may be only uptake of water. In a cell already differentiated and having reached its period of elongation, actual enlargement is therefore dependent, in the first place, on uptake of water by the vacuole, elongation being impossible if this is prevented. ✓ The uptake of water generally is not a limiting factor, however, and this aspect of the process need not be considered here.

✓ In regard to the mechanism of enlargement of the surface of the cell wall, which does concern us here, there are different theoretical possibilities. The enlargement may be initiated:

✓ I. By active increase of cell-wall material, enlargement of the wall being due merely to its active growth, independent of outer forces, as a result of deposition of new substances in the wall.

✓ II. By passive increase of cell-wall material, deposition of new particles in the wall being possible only when there is elastic extension under the influence of turgor pressure. This extension becomes permanent as a result of deposition (interposition) of new particles.

✓ III. By mere plastic stretching of the cell wall under the influence of turgor pressure, the particles of the wall slipping along each other and becoming permanently changed in their positions towards one another.

✓ In other words, energy for enlargement of the cell wall may be derived from active increase of cell-wall material or from turgor pressure. In the latter case the cell wall is extended, reversibly or irreversibly, by the turgor pressure as a result of (a) increased elastic or plastic extensibility of the wall, or of (b) increased turgor pressure itself (greater osmotic value, changed permeability or increased imbibition pressure). In both cases turgor pressure is necessary, however, as a factor in elongation. ✓

All these possibilities have been embodied in theories of different authors, as will be seen from the following short survey.¹

✓ A. *Elastic Extension of the Cell Wall as the Primary Phase of Elongation*

✓ Sachs (1874) was the first to formulate a theory concerning the mechanism of cell elongation. His theory conforms with No. II of

¹ For a more complete survey and references the reader is referred to citation 66.

the above-mentioned possibilities, the primary phase of elongation being elastic extension. According to this author, the mechanism of elongation involves deposition of new particles between those already present in the wall, this being possible only if the distances between particles already present are large enough. Elastic extension of the wall continually renews these spaces, and by interposition (intussusception) of material, the elastic extension is irreversibly fixed, so that the wall will be subject to further elastic extension by turgor pressure.✓

Sachs and later de Vries must be given credit for being the first to have drawn attention to turgor as a necessary factor in elongation, the energy for actual surface enlargement of the cell wall being furnished by this pressure.✓ Their theory has been accepted or rejected by later authors, those who accepted it making a more profound study of the problem whether elastic extension results from increased turgor pressure or from increased elastic extensibility of the wall. Let us consider these two possibilities.

Increased turgor pressure as the initiating factor in elastic extension. De Vries made studies on the osmotic values of opposite sides of organs in growth movements. When he found greater osmotic value on the side of greater elongation, he suggested that elastic extension might be caused by increased turgor as a result of the production of osmotic substances.✓ Krauss (1882), Pfeffer (1892) and Kerstan (1909) did not agree with this view, and did not approve of the experimental data. Krauss (1882), Copeland (1896), Borowikow (1913/14) and Phillips (1920) even found the opposite to be the case. Read (1921), Fernald (1925) and Oberth (1925), who studied the relation between elongation and osmotic value by cryoscopic methods, also came to the same conclusion. Of later investigators, Warner (1928) stated that differences of sugar concentration in the sense of de Vries occur in organs whose geotropic curvature is prevented, but that these differences are accompanied by opposite differences in acid concentration on the sides of the organ. Warner concluded, therefore, that differences in sugar concentration are not the cause of greater elongation during curvature. Ursprung and Blum (1924), studying osmotic properties during geotropic movements of roots, found no differences between the two sides. ✓ In normal roots a different distribution of osmotic values

and of elongation was found, the osmotic value continuously increasing towards the tip. Furthermore, turgor was lowest in areas of greatest elongation. Their conclusions will be more closely considered later.

Some investigators suggested that increased turgor pressure results from increased imbibition pressure of the protoplasm (McDougal, 1916, and collaborators; Walter, 1924).

Tröndle (1910/18), Small (1919) and Brauner (1924), to a certain degree, also accepted increased turgor as a cause of growth movements, the increase resulting from changes in permeability. Although the experimental data of Tröndle have been disproved by Zycha (1928), the possibility of an influence of permeability in elongation remains.

Increased elastic extensibility of the cell wall as the initiating factor in elastic extension. All the investigators cited above considered increased turgor pressure as the prime cause of elongation. That elongation might be initiated also by increase in elastic extensibility of the wall, the turgor pressure remaining constant, was advanced by Laurent (1885) and Noll (1888). The latter showed the difference in elastic extensibility of opposite sides of organs whose tropistic curvatures had been prevented. Later, Horreus de Haas (1928) confirmed these results and concluded that a difference in the elastic extensibility of different parts of cell walls is the cause of different elongation. Morgenstern (1914) and Overbeck (1924) also studied growth curvatures after prevention of tropistic movements, and found the curvature to be completely reversible by plasmolysis in the beginning, but permanent after a longer lapse of time. They also suggested that the first phase in growth movements consists of increase in elastic extensibility of the cell wall.

Contrary to the view of Noll, Wortmann (1887) believed the differences in elastic extensibility to be due, not to qualitative changes, but to the amount of cell-wall material present. According to him, thickness of the wall directly influences and regulates the rate of elongation by changing the elastic extensibility of the total wall, the continually increasing turgor pressure causing continuous extension. Pfeffer (1904) pointed out that the ideas of Noll and Wortmann are both acceptable, pertaining, however, to different cases.

B. *Increase of Cell-wall Material as the Primary Phase of Elongation*

Shortly after formulation of the theory of Sachs that elastic extension of the wall by turgor pressure is the first phase of elongation, opposition arose to that idea. Krabbe (1886) and Schwendener and Krabbe (1893) concluded that the first phase involves active growth of the cell wall, that is, increase in cell-wall material. Later authors, especially Ursprung and Blum (1924), arrived at the same conclusion, for they found that turgor pressure was lowest in areas of greatest elongation. Furthermore, they believed that overstretching of the wall by turgor pressure beyond the point of reversibility does not occur, the force being too small. Hence, according to them, deposition of new material in the wall rather than increase in elastic extensibility is the first phase of elongation.

C. *Increase in Plastic Extensibility of the Wall as the Primary Phase of Elongation*

Schwendener and Krabbe (1893), Pfeffer (1904) and Ursprung and Blum (1924) claimed that extension of the wall by turgor pressure beyond the limit of elasticity does not occur, and this view has been generally accepted. Some few others, however, who made a special study of unicellular material, have described such plastic stretching. Klebs (1888), for one, found that in the alga *Zygnema*, though elastic extensibility of the wall is slight and frequently absent, elongation, nevertheless, is very considerable. He concluded that in some unknown way the protoplast influences the quality of the wall, causing increase in extensibility. From his arguments it follows that plastic extensibility is implied. Later, Lepeschkin (1907) arrived at a similar conclusion from corresponding studies with the alga *Spirogyra*. He studied extension of the cells under different turgor pressures, changing the pressure by placing the cells in solutions of different osmotic values. Extension of the wall was accomplished by increase of turgor pressure. If, under the influence of higher temperature, the turgor pressure was increased beyond its normal value, overstretching of the wall took place. The author pointed to the possibility of this occurring also in normal elongation, but he could not produce any proof. Ziegen-speck (1920) described so-called amyloid substances as having great plasticity and as existing in cell walls. From the presence

of these substances in root hairs he concluded that they probably cause elongation by contributing great plasticity.

D. Condition of the Protoplasm as the Primary Factor in Elongation

Some authors believe that changes in the protoplasm itself may initiate elongation. Such vague ideas, which do not contribute much to our understanding of elongation, were held by Grafe (1920), Borowikow (1913/14) and van de Sande Bakjuysen (1928/30):

E. Criticism

All these studies of elongation have generally been based on coincidence, no other ways being available at the time. From simultaneous changes in elongation and in certain properties, a causal relation was inferred, but very often it was overlooked that with such methods causal relations may never be fully proved. Changes of properties occurring simultaneously with change in elongation may be results as well as causes of elongation, or they may be changes wholly independent of elongation.

II

THE AUTHOR'S THEORY ON THE MECHANISM OF CELL ELONGATION

In 1931 the author of this review formulated the following theory on the mechanism of cell elongation (65-79):

I. The primary factor in cell elongation is plasticity of the cell wall, and the first phase consists of increase in wall plasticity, followed by plastic extension of the wall during which the particles of the wall slide along each other. It is this plasticity (plastic extensibility) which is regulated by the growth hormone.

II. Elastic extensibility of the wall is not a factor in elongation; changes in elastic extensibility are results of actual elongation.

III. Surface enlargement of the wall does not directly depend on production of cell-wall material (active growth of the wall), nor on the degree of elastic extension in the wall.

IV. Energy necessary for surface enlargement of the wall is derived from turgor pressure.

Besides increase in wall plasticity as the primary phase of elongation, many other processes occur in normal elongation, such as

increase of cell-wall material and of other substances, changes in osmotic value and in the properties of the protoplasm, and changes in the elastic extensibility and in other properties of the wall. According to the theory, however, actual surface enlargement of the wall primarily involves plastic stretching of the wall. Plasticity of the wall, therefore, must be considered as the limiting factor in elongation; the other factors, turgor pressure and uptake of water, are generally not limiting, or they are subordinate in so far that actual surface enlargement of the wall is not directly dependent on them, although they generally accompany or follow the first phase. They are probably partially a result of enlargement.

As has already been mentioned, the conclusions of older investigators were based on coincidence, no other means being available, so that it was out of the question to discern which changes were the causes and which the results of elongation. Application of growth substance, however, has made it possible to distinguish between cause and effect.²

The principal experimental data for the theory of the present author may be briefly summarized under the following six headings:

A. Increase in Plasticity of the Cell Wall by the Growth Hormone

The first experiments were carried out with coleoptiles of *Avena*.

In order to concentrate on the first phase of elongation, without the complication of actual elongation itself, growth hormone was applied to coleoptiles severed at their bases and placed in humid chambers. This treatment prevented actual elongation by intercepting the uptake of water. The cutting and application of hormone took place an hour and a half after decapitation, at which moment the quantity of growth substance contained in the plants was at its minimum. Subsequent bending under a weight showed increased plasticity of coleoptiles treated with growth substance as compared with untreated controls, for permanent curvature resulted after bending of the treated plants only. As permanent curvature also resulted after bending at 4° Celsius, growth of the cell wall could not have been its cause. Plasticity caused by such treatment may exceed that of normally elongating plants.

In a later publication by Heyn and Van Overbeek (79) it was shown that, under the influence of growth substance, also the elastic

² For a general survey on growth substance the reader is referred to Vol. 1 p. 162 of this periodical.

curvature of turgescient coleoptiles was somewhat increased beyond that of untreated plants. The difference was much smaller, however, than that in plasticity, and may perhaps have been a result of interaction between different layers of the wall, and not of direct action of the hormone. Moreover, it appeared (67, 68) that by subjecting the untreated plants to a heavier weight, thus causing greater bending, the permanent part of the curvature still remained much less than in the treated plants. Hence, the permanent part of bending is not dependent upon the extent of elastic curvature, as should be the case according to Sachs and others.

B. Over-Stretching of the Plastic Cell Wall by Normal Turgor Pressure

If the severed coleoptiles just described were placed in water, turgor pressure, instead of a bending weight, acting on the walls, the plants provided with growth substance underwent considerable elongation, while untreated plants showed only slight elongation. This elongation was proved to be permanent by comparing elastic contraction on plasmolysis before and after such elongation, the contractions being almost the same in treated and untreated plants. This permanent elongation took place also in water of 1° Celsius, at which temperature increase of cell-wall substances may be supposed to be interrupted.

It follows, then, that turgor pressure may cause irreversible increase in the surface of the cell wall, if only the wall possesses sufficient plasticity induced by the growth hormone. In normal elongation the growth hormone continuously restores plasticity. In other words, the fact that the hormone which regulates elongation influences plasticity of the cell wall before elongation takes place, indicates that elongation is dependent on plasticity of the wall as the primary factor.

C. Decrease in Plasticity of the Cell Wall

There must be decrease as well as increase in plasticity of the wall, for, otherwise, regulation of elongation by the growth hormone would not be possible. This was indicated by the fact that the increase of plasticity induced by the hormone in plants in which elongation was prevented appeared greatest after an action of the hormone during one hour only. After a longer time of action the

subsequent increase in water was smaller, which fact indicates that other processes, resulting in a decrease of plasticity, interfere after a longer time.

The exact nature of this decrease is not yet known, and it may probably arise in several ways. The suggestion originally made (66, 174) was that it should be ascribed to continued cell-wall formation during checked or decreased elongation. Deposition of cell-wall substances, especially of cellulose, makes the cell wall stiffer and less reactive to auxin, probably because the macro-molecules in the wall are better joined together by the new molecules. If elongation does take place, there is a loosening of molecules during plastic stretching. A similar explanation is that also without addition of new molecules the molecules present join together in the course of time, as has been proved for many colloids. Reference is made (73) to the phenomenon of "aging" of colloids, described by Arisz (5), Herzog (61) and Herzog and Koref (62). The same phenomena may probably also occur as a result of extreme plastic stretching, for in that case, too, the macro-molecules finally also approach each other ("Schubverfestigung").

D. Changes in Elastic Extension of the Wall

According to the foregoing data, the hormone which induces elongation first causes increase in plasticity of the cell wall, even if actual elongation is prevented. Since turgor pressure is able to overstretch such plastic walls irreversibly, and since the overstretching may occur at low temperatures, sufficient evidence was available for formulation of a theory according to which the first phase of actual elongation should consist of plastic stretching of the wall by turgor pressure, the hormone regulating elongation by increasing the plasticity of the wall. It was necessary, however, to prove that other possible mechanisms of elongation do not occur.

The new theory is in contradiction to generally accepted theories already mentioned, according to which elongation is dependent on the degree of elastic (reversible) extension of the wall, by which deposition of new particles, between those already in the wall, is made possible. In the new theory emphasis is placed upon the difference between plastic and elastic extensibility, the former being of primary significance, the latter having no significance at all and being a mere result of elongation. Direct influence of the hormone

on both plastic and elastic extensibility, in such a way that both are directly increased, must also be rejected on account of experimental facts to be described below.

Such far-reaching conclusions have made it necessary to study closer the relations between elastic extension of the wall and elongation. The insignificance of this relation as contrasted with that between plastic extension and elongation is indicated by the following observations:

1. As has been previously stated when describing bending experiments, greater artificial bending of coleoptiles not supplied with growth substance does not result in greater permanent curvature than that of coleoptiles containing the substance but subjected to less bending.

2. The rate of elongation of coleoptiles rapidly decreased after decapitation, reaching a minimum after one hour and a half. If at that moment growth hormone was applied, elongation increased considerably, and after one hour equaled that of normal control plants. Elastic extension (measured by contraction on plasmolysis) one hour after application of the hormone was even smaller, after one hour and a half still less, and after two hours only slightly increased beyond the elastic extension at the moment of applying the growth hormone. It never reached that of normal controls.

3. In decreasing elongation by decapitating the coleoptiles (67), the rate of elongation and degree of plasticity simultaneously reached a minimum after one and three quarter hours at which moment they were 0% to 5% of their original values. After that, both increased again, reaching 50% of the original values three hours, and 75% four hours after decapitation. Plastic extensibility of the wall, therefore, closely follows changes of elongation to such a degree that at any moment plasticity and elongation are equal fractions of their original values. Elastic extension, on the contrary, very slowly decreases after decapitation, reaching 90% to 85% of the original values after one and three quarter hours. After this, no increase occurs, but further decrease to 80% four hours after decapitation. Thereafter, increase sets in.

The conclusion is permitted from these facts that elastic extension does not play a contributing part in elongation. The coincidence of the time of minimum elongation and the time of minimum plasticity is in full agreement with what is required for plasticity to be

the prime factor in elongation, and the non-coincidence in time of minimum elongation and minimum elastic extensibility is in full agreement with what would be required for a quality which is a result of elongation rather than a causal factor in elongation.

E. Changes in Elastic Extensibility of the Cell Wall

In order to complete the data obtained on elastic extension, a study of elastic extensibility was pursued, especially to secure some explanation of changes in this property as a result of elongation. The elastic extensibility was determined in plasmolyzed organs. It proved to vary in exactly the same way as elastic extension. After decapitation, the elastic extensibility also reached a minimum in about four hours, at which moment its value was approximately 80% of the original. Subsequently an increase set in, and the original value was attained about five and a half hours after decapitation. Since elongation reached a minimum after one hour and a half, the same conclusion may be made as in the case of elastic extension, namely, that elastic extensibility is not a factor in elongation.

That elastic extensibility and elastic extension are results of elongation and are not influenced by growth substance was proved also by the study of these properties in non-growing plants. If the decrease of both elastic properties after decapitation is a result of decreased elongation³ and not caused by a lack of growth substance, then prevention of elongation by cutting off the plants at their bases should result in their decrease, even though growth substance is sufficiently present and actually causes increased plastic extensibility. This indeed proved to be the case. The extensibility and extension of coleoptiles whose elongation had been prevented by cutting them off at their base, was decreased up to 50% of the original value after 24 hours. Neither the presence nor the absence of the tip, nor of a block of agar containing growth substance, though increasing the plastic extensibility, had any effect on the decrease in elastic extensibility. Therefore, it follows that elastic extensibility

³ In response to the editor's questioning this statement, Dr. Heyn replied: "Your statement that 'decreased elastic extension is the same thing as decreased elongation' is untrue. It is one of the important points emphasized, that elastic extension (not permanent) is quite different from elongation (permanent). Therefore, decreased elastic extension is also quite different from decreased elongation." Dr. Heyn seems to use the word elongation, therefore, not in a comprehensive sense, but only when elongation is permanent. When it is not permanent he does not call it elongation, but merely elastic extension.—Ed.

of the wall is not directly influenced by growth substance but is altered only as a result of change in the rate of elongation. Since under the same conditions plasticity is increased by growth substance, plastic and elastic extensibility should be separated in regard to their significance in elongation.

At 0° Celsius decrease in elastic extensibility and extension upon prevention of elongation did not occur at all, which fact suggested that such decrease is caused by some special physiological or colloidal process.

Besides the decreased elastic extensibility just described, also increase of elastic extensibility shows itself in certain cases, for instance, 4 hours after decapitation, when the decrease is followed by an increase. Hence, two interfering processes influencing elastic extensibility occur in normal elongation, a process of increase, predominating if the rate of elongation is accelerated, and a process of decrease, predominating if elongation is inhibited.

F. *Experiments with Other Materials*

All experiments described above were carried out with coleoptiles of oat seedlings, the classical material which served for the first experiments on the regulation of elongation by the growth hormone. Growth consists here solely of cell elongation without cell division (compare Tetley and Priestley and later Avery). For a general theory of elongation, however, it was necessary to add experimental data obtained with other material, and quite the same results were obtained (73) with hypocotyls of *Lupinus*, flower stalks of *Tulipa*, and internodes of *Vicia Faba*. The experiments in these cases were also carried out under prevention of actual elongation. The bending was produced by submitting the organs to a constant and fairly intense mechanically applied bending which gradually resulted in permanent curvature. This method enabled determination of plasticity within five to ten minutes. With these materials experiments were also carried out on the influence of geotropic stimulation on the plasticity of the cell wall. Sections of hypocotyls of *Lupinus* were exposed to geotropic stimulation while in a moist chamber, under which circumstances no curvature occurred, owing to the prevention of water intake. The first differences in plasticity of opposite sides were observed after exposure of half an hour. This is in agreement with the data obtained by Dijkman (47) who with

the same material showed that the first differences in distribution of growth substance over the two sides of the hypocotyls also occurred after the same length of time.

It is of importance to note that in a similar manner as with growth substance, the plasticity also proved to be distributed over the two sides in such a way that increase of plasticity on one side corresponded to decrease on the other, the total amount remaining the same during the process. Furthermore, it was possible to completely reverse the difference of plasticity in the two sides by merely submitting the organ to stimulation in inverse position, *i.e.*, by turning it over 180 degrees, so that the former lower side became the upper. This would make it appear rather improbable that increase in plasticity is a result of any formation of wall material, as such a process can not be expected to be reversible in such a way. The growth substance proved to be redistributed in a similar manner.

The influence of temperature on plastic stretching of the cell wall was very slight and of the same order as in colloids or other non-crystalline substances. The temperature coefficient proved to be 1.2, which is much smaller than the Q_{10} of most vital processes. The nature of the influence of temperature on plasticity of the wall is probably different from that of the action of growth substance, the former increasing plasticity by influencing the heat movement of molecules, the latter by influencing the structure of the wall.

The resulting stretching was proportional to the strain applied. The experimental results altogether indicate that plastic elongation of cell walls is of a character similar to the stretching of colloidal matter, and not of the character of a vital process.

III

NON-CORROBORATING DATA AND CONCLUSIONS OF LATER INVESTIGATORS

Horreus de Haas (1928) was the first to claim that elastic extensibility of oat seedlings decreases after decapitation, although his experimental material was not sufficient to substantiate the conclusion. From his experiments he concluded that growth substance probably influences the elastic extensibility (called elasticity by him) of the cell wall, which, according to him, would be in agreement with the ideas of earlier investigators (Sachs) who also considered elastic extensibility of the wall as the first factor in elongation.

Söding (135) came to a similar conclusion. His first publication appeared shortly after that of the author of this review. He also established differences in elastic and plastic extensibility of normal and decapitated coleoptiles, hand in hand with differences in extensibility. In later experiments with flower stalks he (137) found that in these organs, too, decapitation caused decrease of both elongation and elastic extensibility, but not of plastic extensibility. Furthermore, he found that the plasticities of normally growing flower stalks, as obtained with his methods, were very variable in different stalks, whereas the rates of elongation were rather equal. From these facts Söding concluded that plastic extensibility is of no importance whatever as a factor in elongation. He believed the changes in plasticity to be results of elongation. His results differed from those of the present author, obtained with flower stalks of *Tulipa*, where clear differences in plasticity were observed (73).

The methods applied by Söding were different from those described in the foregoing chapter, for his experiments were carried out only with elongating organs (no experiments were pursued with organs in which elongation was prevented) which were not suitable for causal analysis. It is impossible to distinguish between the influence of plastic and of elastic extensibility under such conditions, and the question was wisely left open by Söding as to whether the changes of properties described were to be interpreted as causes or as results of elongation. Furthermore, the flower stalks were treated with boiling water before determination of plasticity, a treatment which most certainly affects the cell wall. Söding himself admitted this point. In addition, the turgescient organs were bent for 15 seconds only, under very slight weight. This time and weight are certainly too small to obtain irreversible curvature, for, as has been stated in the foregoing chapter, the first stage of plastic bending always shows itself in increased elastic curvature.

In another report Söding (136) showed that the first stages of curvature of coleoptiles under the influence of growth substances are irreversible on plasmolysis. This fact may be explained by accepting plastic stretching as well as active growth of the cell walls. Studying the plasticity of the two opposite sides of the same coleoptiles, Söding (138) found, furthermore, that the curvature reached a constant angle after $2\frac{1}{4}$ hours, while during at least $3\frac{1}{2}$ hours the plasticity of the convex side remained 35% larger than that of the

concave side. At first sight his conclusion that plasticity must therefore be a result of elongation seems rather acceptable, and if it were also true that plasticity reached a minimum later than elongation when elongation was in a straight line, his conclusion would be wholly justified. In the foregoing chapter experiments have been described, however, proving that in straight elongation plasticity closely parallels changes in the rate of elongation, both reaching a minimum at exactly the same moment.

The different conclusion of Söding can be easily explained by the fact that in his case differences in rates of elongation (angle of curvature) were compared with ratios of plasticity. If the data of Söding are considered for each side separately, taking the figures of the absolute plasticity instead of those calculated for the ratio of plasticity of the two sides, other relations appear, plasticity and elongation appearing to reach a minimum simultaneously in his case also. Thereafter increase again takes place. It is true that the concave side continuously has less plasticity than the convex side and that after the curvature becomes constant both sides have different plasticity and an equal rate of elongation. This can easily be explained, however, by the greater osmotic value and turgor pressure which of necessity must be present in the cells of the concave side as a result of less elongation during the previous two hours of curving. Although the cell walls of the concave side have less plasticity, their greater turgor pressure induces plastic stretching similar to that on the convex side. But on the concave side there is no question whatever of any cessation of elongation after two hours, and constant plasticity after elongation has already reached a minimum.

Thus the experiments of Söding do not prove that plasticity plays a part in elongation,⁴ and his further conclusion that active growth of the cell wall must be the first phase of elongation—being based on his assertion that plasticity plays no part, without any direct experiments to prove it—is also not substantiated.

⁴ In further explanation of this statement the author wrote: "A consideration of the results of his experiments just described *might* suggest that also plasticity plays a part in elongation and even Söding did not completely exclude this possibility in his publication, contrary to his later opinions. I want to say that his experiments are not conclusive and do not prove that either active cell-wall growth or plastic extension plays a part in elongation." [Ed.]

Similar data which on close inspection are not in contradiction to the conclusions of chapter II were described by Zollikofer (157). This authoress studied the extensibility of cell walls on the dorsal and ventral sides of flower stalks of *Papaver* and *Tussilago* during floral movements. It was established that during bending about 80% of the difference in length of both sides was due to greater elastic extension of the dorsal side, and only 20% to its greater permanent length. During subsequent unbending of the flower stalk, only 20% of the length difference was due to elastic extension. During bending the greater elastic extension of the dorsal side was caused by greater elastic extensibility, both plastic and elastic extensibility being increased on this side. During unbending the plastic and elastic extensibility of the ventral side was greater. Although the presence and action of growth substance were not studied, Zollikofer suggests that the great elastic extensibility, occurring during the bending, is caused by this substance. Referring to chapter II and VIII, the greater elastic extension during bending of the flower stalk can be explained also as a result of continuously greater plastic stretching of the walls on the same side.⁵

Another author whose data can perhaps suggest conclusions different from that stated in the foregoing chapter was Strugger (143). He made a study of the influence of acids on elongation by comparing the rate of elongation and viscosity of the protoplasm as determined by velocity and shape of plasmolysis. He found parallelism between them in so far that cells of greater elongation always showed greater viscosity of protoplasm. Strugger concluded, therefore, that the first phase of elongation is an increased swelling of protoplasmic colloids. The altered state of protoplasm would result from the action of acids formed by increased respiration. According to him, the changed properties of protoplasm directly influence elongation by the resulting increase of imbibition pressure; or indirectly by causing a change of cell-wall properties.

Bonner (12) interpreted Strugger's result as an influence of acids on the dissociation of growth substance, the latter being a weak acid of which the salt form is inactive, and the dissociated form active.

⁵ The editor's comment at this point prompted the following response: "Greater elastic extension can be indeed a result of (greater) plastic stretching. As a result of plastic stretching (which results in a permanent increase of length) the cell wall becomes thinner and therefore gets greater elastic extensibility. During plastic extension the cell wall is at the same time also in a state of elastic extension." [Ed.]

He showed close parallelism between the rate of elongation and that of dissociation of the growth hormone as affected by different pH values of the tissue. By decreasing the pH the increase in rate of elongation was closely parallel to the amount of non-dissociated growth substance. These experimental results were recently confirmed and amplified by van Santen (133).

Albaum and co-workers (compare 133) found that penetration of heteroauxin into the cell also depends on its dissociation, and pointed out that in this way, too, the influence of pH on elongation can be explained. Their conclusion has as much ground as Bonner's, all being based on coincidence. The fact that the influence of pH remains the same also when no growth substance is present in the outer solution, makes Bonner's conclusion the more probable.

The possibility of direct action of acids on properties of the cell wall without intermediation of protoplasm or growth substance, has been mentioned by Brecht (22) who quite rightly pointed out that equivalent effects of growth substance and acids on elongation does not imply that the action of growth substance must be interpreted as an action of acids on the protoplasm (as by Strugger), or, conversely, that action of acids must be interpreted as an action of growth substance (as by Bonner).

✓ Ruge (129) confirmed Strugger's experimental data but showed that increase in viscosity of protoplasm occurs only after 18 to 42 hours, whereas corresponding increase of elongation occurs after one hour. By this fact it is definitely proved that change in viscosity of protoplasm can not be the first phase of elongation.

The protoplasmic differences found by Strugger can be explained in two different ways. They can be mere results of elongation, or they can be caused by the growth hormone. In the latter case, however, they are influenced independently of the effect of growth substance on the first phase of elongation, occurring at a much later period. They might be the first phase of other phenomena arising from action of the hormone, as, *e.g.*, root formation.

In all events, without considering the role played by the protoplasm, the pH, according to Bonner (1934) and Ruge (1938), always influences elongation by its direct or indirect action on plasticity of the wall, and these data are in full agreement with the theory given in the previous chapter.

Czaja (43, 44) carried out investigations on plasmolysis (bursting) of root hairs in sugar solutions, and found that at the same pH indole acetic acid had a somewhat greater effect on the increase of plasmolysis than HCl. From his conclusion that growth substance changes the electrical charge of the cell membrane, resulting in increased turgor pressure, there follows a rather elegant hypothesis on the action of the growth hormone in his case. But even if his hypothesis were proven by the experimental data it may not be taken for granted that the action of the hormone on elongation also occurs in that manner. A dual action of the hormone is quite possible, protoplasm on the one hand, and the cell wall, on the other, being independently influenced.

The significance of increased turgor pressure as a primary limiting factor in elongation was opposed by Ursprung and Blum, and Ruge (129, 130) has recently proved by direct experiments that the growth substance indole acetic acid does not influence osmotic pressure in normal elongation. In addition, Thimann and Bonner (149) state that the amount of growth substance causing distinct elongation is not sufficient to cover even part of the surface of the protoplasm in a monomolecular layer.

Another author who ascribed significance to osmotic pressure as a primary factor in elongation, was Friedrich (53). Confirming the experimental data of Warner (1928) and Metzner (103), this author found that after geotropic stimulation and simultaneous prevention of actual curvature, a higher amount of reducing sugars is present in the lower than in the upper side of seedlings of *Helianthus*.

His conclusion that this difference accounts for the difference in elongation between both sides, can not be accepted, as it was not proved that the change in sugar content took place at the same moment that change of elongation between both sides occurred. Moreover, differences in osmotic value did not occur on application of indole acetic acid. Besides, Warner (1928) showed that the changes in sugar concentration are accompanied by opposite changes in acid concentration on both sides.

IV

CORROBORATING DATA AND CONCLUSIONS OF LATER INVESTIGATIONS

Plastic properties of cell walls were described by some of the early investigators (Klebs, 1888), but after Pfeffer (1893) em-

phatically denied the possibility of the wall becoming over-stretched by turgor pressure, the idea was generally abandoned and the study of plasticity lost interest. Only a few authors (Overbeck, 1926) gave it attention in the following years. Soon after formulation of the new theory (chapter II), however, plastic stretching by turgor was again detected by several investigators (Pringsheim, 1931; Martens, 1931; Overbeck, 1934; Schoch Bodmer, 1936). All of them agreed in emphasizing that the idea of over-stretching by turgor differed from what had been generally conceived.

Pringsheim (125) showed that prolonged immersion in water, even at low temperature and after plasmolysis, of strips of hypocotyls of *Helianthus* and strips of potato, resulted in permanent elongation, as concluded from plasmolysis before and after immersion. He concluded that the walls were over-stretched by turgor pressure, and disputed Pfeffer who denied this. Pringsheim suggested that we speak of growth only if simultaneously with surface enlargement new particles are added to the wall. In his experiments at low temperature, or after transitory plasmolysis, this was probably not the case. According to the theory given in chapter II, both plastic surface enlargement of the wall and increase of cell-wall material occur during elongation, plastic stretching directly causing actual surface enlargement, while increase of cell-wall material occurs for the most part simultaneously and probably independently, in exceptional cases being totally absent perhaps.

✓Martens (100) described rapid plastic elongation of staminal hairs of *Tradescantia* up to 250% of the original length, commencing after release upon bursting of resistance offered by the cuticle. Overbeck (116), using the same methods as Pringsheim, demonstrated over-stretching of the wall by turgor pressure in the rapidly elongating seta of the sporogonium of *Pellia*. Within a few days, increase in length up to 9000% took place. In this connection it is of interest to note that thickness of the wall showed clear decrease during elongation. According to a calculation of Overbeck, the surface of the cell wall increased 31 times, whereas the volume of the walls increased only 5 times during the same period of elongation. It is evident, therefore, that elongation occurred mainly at the expense of cell-wall material, and that plastic extension greatly exceeded deposition of new material. Elastic extension, on the other hand, was differently distributed, with no apparent causal relationship between it and the rate of elongation. These data are in agree-

ment with the theory of chapter II, although growth substance could not yet be detected in the seta. With regard to the possible absence of growth substance in this case it must be emphasized that the new theory does not claim the presence of the hormone for all cases. The hormone is not necessary if the cell wall has sufficient plasticity without it. Schoch Bodmer (1934), quoted from Frey (1934), in similar ways demonstrated plastic stretching by turgor pressure in the filaments of grasses.

Märkert (1931) and Böhner (1931/33) studied plastic properties and plastic stretching by turgor during thermonastic movements of *Tulipa*. Elastic extensibility of the cell walls in petals proved to be very small, plasticity, on the contrary, being very large. Märkert ascribed the plastic properties to the amyloid nature of the cell wall. Böhner, determining the plasticity at different temperatures and at different stages of the movement, claimed that the movement might be a result of different plasticity in the walls of opposite sides. He concluded that the mechanism of thermonastic movement completely agreed with the mechanism of elongation as described in chapter II. Michaelis (1934) finally pointed out the significance of plasticity in the movement of tendrils.

The authors referred to above studied plasticity of cell walls and plastic stretching irrespective of growth substances; the authors to be mentioned in the following studied it in connection with growth substance.

Gessner (55, 56, 57) compared the influence of various factors on elongation and plasticity of cell walls in hypocotyls of *Helianthus*. He stated that, in general, all factors decreasing plastic extensibility also decrease elongation (56). The factors investigated were: decapitation (decrease in amount of growth substance), acids, ordinary and ultra-violet light. Increase of growth substance and changes in pH (up to 4 or 5) increased both elongation and plasticity. After decapitation plasticity immediately decreased, elastic extensibility showing decrease only after 4 hours and remaining constant during restricted phototropic curvature. The plasticity of the cell wall showed great differences in the same cases. These experimental data are in full agreement with the ideas given in chapter II.

At first sight, one single experimental fact described by Gessner—concerning the influence of temperature on elongation and plas-

ticity—seems to differ from these ideas. When he kept his plants at a higher temperature for 18 hours, plasticity of the cell walls, as determined after plasmolysis, was found to have decreased, elongation during the same period having increased. He endeavored to explain this by pointing to the likelihood that elongation is dependent on other factors in addition to cell-wall plasticity. According to him, temperature would increase these other factors, the final result being increased elongation in spite of decreased plasticity. This hypothesis is very attractive, and it is even quite certain that elongation is dependent on many other factors besides plasticity, the latter generally being the limiting factors, but the experimental results can be explained also in a much simpler way. In the foregoing chapter it has already been clearly shown that plasticity of cell walls is increased by higher temperature. During these experiments actual elongation was prevented, as otherwise elongation would have interfered with plasticity. The diverging conclusion of Gessner, that plasticity of the wall is decreased by higher temperature, must be ascribed to the fact that in his experiment the plants underwent elongation during the 18-hour exposure to higher temperature. The higher temperature permits temporarily greater plasticity as a result of physical changes in the wall. This greater plasticity will be used by plastic stretching if actual elongation takes place. This disturbance of the original equilibrium between plasticity and plastic stretching will result in a decreased plasticity when the plants are returned to the original lower temperature. Gessner did indeed find gradual restoration of the original plasticity when the plants were returned to the original lower temperature, as must be the case when a return to an original equilibrium takes place. If the phenomenon was caused by direct influence of the temperature on the properties of the cell wall, an immediate change in plasticity would be expected when the plants are returned to a lower temperature.

Thimann and Bonner (149) and Bonner and Thimann (17) studied the quantities of growth substance entering into and disappearing from the plant (extraction of growth substance with ether). The growth of coleoptiles proved to be proportional to the amount of growth substance entering the plant, and also to the amount used in the plant. When an excess was applied, or elongation prevented, the excess disappeared and there was not any excessive growth.

The relation between growth substance entering the plant and cell-wall material produced as a result of its action was studied more closely. According to calculations of these authors, one molecule of growth substance acts in the laying down of 3.10^5 $C_6H_{10}O_5$ residues, or of about 140 cellulose micelles (if a cellulose micelle in coleoptiles is accepted as consisting of 2000 glucose residues). They conclude that growth substance does not play any stoichiometric part in deposition of cellulose or of the total wall. This is in full agreement with chapter II, where production of cell-wall substances is concluded not to be influenced by growth substance.

Also, growth substance does not act by producing a monomolecular layer upon the new cell wall laid down, according to further calculations of Thimann and Bonner. In a particular case the area of new cell wall laid down was 40 mm.², whereas the area covered in a monomolecular layer by the amount of growth substance causing the increase of cell-wall surface was calculated to be only 0.3 mm.². The authors, therefore, conclude that it is very improbable that growth substance acts by influencing the permeability of protoplasm.

From the claim in chapter II that growth substance does not directly influence elastic extension of the wall, it was concluded by the author of this review that osmotic value of the cell, permeability of the protoplasm, and elastic extensibility of the wall are not directly affected by the hormone. Ruge (129, 130) studied the action of growth substance on these properties, and completely confirmed the results obtained in the indirect way. The material studied was the hypocotyl of *Helianthus annuus*, and the growth substances used were indole acetic acid, phenyl acetic acid and phenyl propionic acid, dissolved in lanoline according to the method of Laibach. The paste was applied on the tip of the decapitated hypocotyl, giving lengthwise diffusion through the hypocotyl; or, it was applied as a ring round the zone of elongation slightly below the tip, giving diffusion in a transverse direction. It was found that elongation always took place in the direction of diffusion of the hormone. In the first case, the lengthwise walls elongated, whereas in the second case only the transverse walls did so. The author concluded that transport of growth substance takes place within the walls themselves.

Under the influence of growth substance changes in the properties of protoplasm in the elongating cells were also proved to occur.

Increase in viscosity, as determined from the speed with which the rounding of the protoplast in plasmolysis occurred, and decrease in permeability for chrysoidin, neutral red and ureum, were found, but not before 18 to 42 hours after application of the growth substance. As these properties changed much later than elongation—elongation changing immediately after application of the growth substance—it was concluded that they have no bearing as a cause of elongation. According to Ruge, the properties of protoplasm can be affected presumably by action of the hormone independently of its action on elongation. It was suggested that the hormone, present at first in the wall changing in plasticity, afterwards enters the protoplast and thereupon changes the viscosity and other properties of the latter. The osmotic value of the cells, furthermore, decreased immediately if elongation was accelerated. This was interpreted as a direct result of the elongation caused by increased uptake of water. As soon as dilution has occurred to some extent increased production of osmotic substances commences. This latter process was found to consist of transformation of bioses into monoses, to begin with, and later of increased uptake of salts into the cell. In the beginning, the diluting process predominated, so that the osmotic value first decreased, to become constant afterwards. When elongation was checked, both the amount of osmotic substances and the osmotic value increased. Plasticity of the walls was directly and immediately increased by the growth substance, and Ruge completely agrees with the theory given in chapter II that plasticity of the cell wall is the primary factor in elongation.

V

EXPERIMENTS WITH ROOTS

At the time of the first investigation described in chapter II, the presence of growth substance in roots had not yet been detected, so that analysis of elongation in roots, as had already been described for other organs, was not possible. In some experiments on the plasticity of roots, the conditions in these organs, furthermore, appeared rather complicated. For these reasons the conclusions of chapter II were confined for the time being to shoots and other negatively geotropic organs, and the questions whether in elongation of roots growth substance plays a part, and whether the mech-

anism of elongation of roots and the action of growth substance in them is the same as in shoots, were left open (66, 226).

After indications of the possibility of hormonal action in elongation of roots were obtained by Cholodny (1928) and more definitely by Hawker (60), Boysen Jensen (19) first isolated the growth hormone from them. His data were confirmed by Thimann (146) who also extracted the substance from roots, using chloroform or ether. The author of this review (74) proved the growth substance of roots to be identical to that of shoots.

That growth substance has an influence on elongation of roots followed from researches on the action of solutions of the substance on elongation of roots, and from the redistribution of the substance during geotropic stimulation of roots.

Hawker (1932) and Boysen Jensen (21) first proved that distribution of growth substance in roots on geotropic stimulation takes place in a similar way as in shoots, the lower side obtaining a greater amount than the upper side. Boysen Jensen (20, 21) showed, furthermore, that the ratio of growth substance after distribution on geotropic stimulation corresponds exactly with the inverse ratio of elongation of the two sides. Therefore, growth substance in roots must inhibit rather than accelerate elongation. This was proved by Nielsen (113), Navez (141) and Boysen Jensen (20) who showed that elongation in roots was retarded when they were placed in a solution of growth substance obtained from *Rhizopus*. The same was shown by Kögl, Haagen Smit and Erxleben (1934), making use of auxin *a* and *b* and indole-3-acetic acid, and by Thimann (147) with indene-3-acetic acid. Inhibition of elongation proved to be proportional to concentration of the growth substance, and not to be a result of change in pH caused by it. [Compare Faber (40) and Marmer (99)]. The fact that growth substance inhibits elongation in roots but accelerates it in shoots, at first sight, therefore, suggested a different function in roots.

The inhibiting action there, however, proved to change into an accelerating action if very low concentrations of the substance were present. This was proved for the first time by Amlong (1936) who showed that in roots elongation is increased by the action of 10^{-9} moles of indole acetic acid, if only the roots contained little growth substance before. Thimann (1936) arrived at a similar conclusion in experiments where growth substance was applied to

the base of the stem while its influence in the root was studied. In cultures of isolated roots, Fiedler (49) showed that 2.10^{-9} moles of indole acetic acid caused 30% acceleration in elongation, whereas higher concentrations caused inhibition. Geiger-Huber and Burret (54) obtained similar results.

It is of significance, also, that in shoots very high concentrations of growth substance also cause inhibition. An optimum action curve of growth substance in *Avena* coleoptiles was described by Jost and Reiss (1936). In roots, furthermore, equal concentrations can cause opposite reactions in different material (146, 48).

It is most probable, therefore, that the function of growth substance is similar in all cases, but that the sensitiveness of cells is different (compare Boysen Jensen, 1936a). Thus the action of growth substance can be represented by an "optimum" curve (convex curve), the optima in different cases corresponding to various concentrations, lower in roots, higher in shoots. Since the concentration normally present in organs corresponds with a point on the action curve after the top has been reached in the case of roots, and with a point before the top has been reached in the case of shoots, it must follow that any increase of concentration causes inhibition of elongation in roots, and acceleration in shoots, decreases in concentration causing opposite reactions. Nevertheless, in both cases growth substance is necessary for elongation, and always acts in a similar way, causing increase in size of the cell. This explanation is quite different from the conclusions of some authors that in roots growth substance causes absolute inhibition of elongation. The opposite geotropic curvature of roots and shoots— notwithstanding the same distribution of growth substance over the two sides upon geotropic stimulation—can be explained by this hypothesis which is in accordance with all experimental observations.

If the total amount of growth substance present becomes too small elongation of roots decreases just as in shoots, and finally ceases altogether if there is no more growth substance at all. Fiedler (49), whose experimental data showed that growth substance could not be obtained from isolated roots cultured under sterile conditions, disagreed, however, with the idea that some growth substance must be present in roots to enable any elongation. He concluded that under certain conditions roots will elongate in

the total absence of growth substance. Nagao (108, 109, 110) proved later not only that in the same kind of roots, as studied by Fiedler, growth substance is present, but also that elongation of such roots and the presence of growth substance always go hand in hand. If the roots were cultivated on plain agar they ceased to elongate, and at the moment elongation stopped, no more growth substance could be detected. If the same roots of stopped elongation were thereupon transferred to the nutrient agar they regained growth substance at the same moment that elongation recommenced.

At present nothing is known of the nature of the difference in the sensitivity of roots and shoots towards growth substance. Presumably, the electrical charges of colloidal particles of the cell wall or other physical chemical properties, as well as structural properties of the wall, play a part.

If the function of growth substance is the same in roots and shoots, the first result of its action, namely, increase in plasticity, must also prevail in both cases. Indeed, Amlong (1) found decreased plasticity of cell walls after decapitation of roots, exactly as described in chapter II with regard to shoots.

The apparent contradiction referred to by Jost (87, 88) between increased plasticity in decapitated roots (as described by Amlong) and inhibition in roots by growth substance can be fully explained by supposing that in the cases referred to the concentrations of growth substance were different.

Czaja (42, 43, 44), also taking a similar function of growth substance in roots and shoots for granted, tried to explain the opposite reactions in them as being due to the interference of a stream of growth substance coming from the tip with another coming from the base of the root. Separately, each stream promoted growth, but combined, they retarded elongation. Faber (48) and Thimann (146) disapproved of this theory, however.

Summarizing, it may be concluded that the function of growth substance is most probably the same in roots as in shoots, and that the mechanism of elongation is also the same in both cases, taking place as described in chapter II. The only difference between roots and shoots involves the greater sensitiveness for growth substance in the former.

VI

FORMATION OF CELL-WALL MATERIAL AND ITS RELATION
TO ELONGATION

The expression "increase of cell-wall material" will be used in the following to indicate any such increase without taking into consideration whether it occurs by apposition, or by intussusception of new particles in the wall. It consists of two stages, formation of cell-wall material and subsequent deposition in or on the wall.

Plastic stretching of cell walls was concluded by the present author to occur independently of formation of cell-wall material. This was deduced from the following facts: (a) Irreversible elongation of coleoptiles in water under the influence of growth substance took place at 0° Celsius, at which temperature formation of cell-wall substances may be supposed to cease (66); (b) Plastic bending, under the influence of weights, of organs containing growth substance took place at low temperatures (79); (c) Growth curvatures caused by growth substance applied on one side were described by Friedrich (53) as taking place at 0° Celsius. (d) Since increase in plasticity of walls is reversible, it appeared very improbable that increased plasticity depends on increased production of cell-wall material preceding plastic stretching (compare Söding, 138). (See chapter II, F (p. 16) and 73).

From all this it was concluded that surface enlargement of the wall is not directly caused by production of new wall material (66), but that it takes place independently of wall formation, and that both processes are independent of each other. Nevertheless, they can occur simultaneously, sometimes the one, sometimes the other, predominating. Generally, they are in concordance with each other in such a way that the cell-wall material per surface area increases as much by deposition of new material as it would decrease as a result of plastic stretching. (Compare 66, 197.) This was proved by Bonner (13) in determinations of cell-wall material. This author showed, however, that elongation is not of necessity attended by a corresponding amount of cell-wall formation. He compared the dry weight of walls of coleoptiles with their elongation while they were immersed in water and in a solution of growth substance. At 25° Celsius the dry weight closely paralleled elongation, the ratios of elongation and dry weight of cell walls being

exactly the same in both cases. At 0° Celsius, however, considerable elongation took place in the coleoptiles placed in the solution of growth substance, but not increase of cell-wall material, so that elongation exceeded cell-wall formation. On the contrary, if the organs were placed in a solution of growth substance to which 1% fructose had been added at 25° Celsius, formation of cell-wall material exceeded increase in length. At 0° Celsius, the fructose had no effect upon cell-wall deposition. This fact makes it very likely that cell-wall formation is checked at low temperatures, a supposition which has already been accepted in this review.

The experimental results of Overbeck (116) also bear out the conclusion that surface enlargement of the wall and increase of wall substances are independent of each other. This author found that within a few days the increase in length amounted to 3,100% during rapid elongation of the seta of the sporogonium of *Pellia*, whereas increase in volume of cell walls amounted to only 500%. Elongation, then, largely exceeds formation of cell-wall material.

It follows that both processes may or may not compensate each other at normal temperatures in the same area of an organ. Ruge (129) studied these relations more closely. He determined the thickness of cell walls from their double refraction and found thinner walls in the upper part than in the lower part of the elongating zone of the hypocotyl in *Helianthus*. The thickness of walls in the upper part decreased the same percentage as length increased during a definite time of action of the growth substance; so it was concluded that only plastic stretching occurred. In the lower part an increase of wall thickness was observed. Ruge, therefore, originally concluded that surface enlargement of the wall and deposition of new material must be separated, not only as primary and secondary phases in elongation, but also with regard to time and place. The two processes occur at different times, and also at different places, plastic stretching without formation of cell-wall material in the upper areas, and increase of cell-wall substances without plastic stretching in the lower areas, of elongation. He further states that in one case, the one, in the other case, the other process is the direct cause of elongation. This theory unites the ideas of surface enlargement of the wall by plastic stretching and by intussusception, both being supposed to occur as two different phases, each of which can independently cause elongation. Later, Ruge

(130, 357) described plastic stretching as taking place also during the second phase of elongation. If this is so, his experimental data quite agree with the theory of chapter II, and it is highly probable that surface enlargement of the walls during the second phase can be satisfactorily explained by plastic stretching only, intussusception occurring independently. The different thicknesses of cell walls in the upper and lower areas of the elongating zone can then be fully explained as results of the different ratios between plastic stretching and deposition of cell-wall material in the different zones. The latter process may even be completely absent from the upper areas and predominate in the lower, but this does not necessarily imply that deposition of cell-wall material in the lower areas is a cause of elongation, too. Anyhow, according to Ruge, the latter process would not be influenced by growth substance, for he found increase of the thickness of cell walls after decapitation not to be controlled by growth substance. With regard to his methods, it must be noted that all results were obtained on elongating organs, so that all changes of properties observed can be interpreted also as results of elongation.

VII

THE POSSIBILITY OF PROTOPLASM AS INTERMEDIARY

In the present author's theory the question whether growth substance acts indirectly by intermediation of protoplasm or directly on the cell wall, was left open. The close relation between protoplasm and cell wall (Wiesner, 1886), on the other hand, and the influence of transient plasmolysis on elongation (Reinhard, 1899), on the other, was only mentioned in this connection. It was concluded that the wall must be considered more as a living organ than as a dead structure of the cell. Reference was also made to investigations of Hansteen Cranner (1914, 1926), according to which the phosphatid components of the protoplast merge into the wall between its micelles. Bonner (14) also considered this question.

The slight concentrations in which growth substance acts was the reason for several authors to suppose an indirect action of it through intermediation of the protoplasm. Especially Gessner (1934), Bonner (14) and Brecht (22) emphasized this.

If the action takes place in this indirect way, it is presumable that special metabolic processes of the protoplasm are intermediately

influenced, respiration being the first to be affected. However, Boysen Jensen (1925) found no difference in respiration of normal and decapitated roots; neither did Nielsen and Hartelius find any influence of the growth substance of *Rhizopus* on respiration of *Aspergillus niger*. Bonner (16), on the contrary, noted an influence of the growth substance of *Rhizopus* on the respiration of coleoptiles. He found that elongation stopped in the absence of oxygen, and that both respiration and elongation were inhibited by the same concentrations of KCN. Brecht (22) later also noted complete inhibition of elongation of coleoptiles in the absence of oxygen; if the oxygen was decreased to 2½% no influence was noticed. In hypocotyls of *Helianthus annuus*, however, complete absence of oxygen did not entirely stop elongation. Bonner found, further, that both respiration and elongation of coleoptiles were increased up to 30% by the growth substance of *Rhizopus*. He concluded that either increase of respiration is a real component of the action of growth substance, or the phenomenon is only an accompanying one, caused perhaps by impurities of the growth substance used. In fact, von Hulssen (80) and Kögl, Haagen Smit and Hulssen (92), using pure growth substance instead of the impure product of Bonner, found no influence at all on respiration. This was acknowledged later by Bonner himself (16). Finally, the data of Pratt (121) can not be interpreted as proving an influence of growth substance on respiration.

Strugger (143), whose investigations on elongation have already been mentioned, deduced from his experimental data the hypothesis that either growth substance acts directly on the protoplasm, increasing its viscosity, the increased imbibition pressure of the protoplasm directly causing elongation, or that the resulting changes of protoplasm cause it to influence the cell-wall properties. He suggested that changes of protoplasm in their turn are results of increased acid concentration of the cell caused by increased respiration.

It is clear from these data that the first action of growth substance does not involve an influence on respiration. The fact remains, however, that elongation is checked when respiration is completely stopped. It is certain, therefore, that somehow some function of living protoplasm, as, for instance, formation of cell substances, must be secondarily involved in elongation, but this does not mean

that action of growth substance on the wall must take place by intermediation of protoplasm.

Gessner (56) and Ruge (129) are the only authors who carried out experiments in order to settle the question whether changes in cell-wall plasticity occur through the protoplasm or not. Gessner, studying the action of ultra-violet rays on extensibility of cell walls and on elongation, found that both were simultaneously decreased. This did not occur if the cells had undergone transient plasmolysis, or had been killed by previous boiling. He concluded, therefore, that either ultra-violet rays influenced plasticity of the wall through the protoplasm, or that plasmolysis and boiling water caused such changes in the wall that there was no more sensitiveness towards growth substance. Brecht (22) also accepted an indirect action of growth substance. Ruge (129), carrying out similar investigations as Gessner, arrived at different results. He found that the growth substances indole acetic and acetic acids considerably increased extensibility of cell walls, even if the cells had been previously plasmolyzed or treated with boiling water or narcotics. Under the influence of these substances, plasticity of the dead walls continuously increased. Sodium hydroxide and sodium acetate, on the contrary, caused decrease in plasticity. The different results of Gessner were ascribed by Ruge to the small range of concentrations investigated. Ruge's observation that the internal pH of cell walls is by no means the same as that of the external solution is also of interest in this connection. Ruge concluded that growth substance can directly influence extensibility of walls without intermediation of protoplasm. According to him, the possibility of direct action of growth substance on protoplasm must not be rejected, however. Were that the case these changes of protoplasm must then occur separately from action on the wall, for they were noticed as not taking place until 18 hours after application of the growth substance, whereas action on the wall took place immediately. The hypothesis was made by him that growth substance is first present in the wall, directly causing increase in plasticity, and that it thereupon enters the protoplasm where it probably can also cause changes; but Ruge left the latter question open.

Robbins and Jackson (128) also investigated the influence of growth substance on dead cell walls, and found increase of extensibility in all kinds of shoots; in living or dead roots, however, they

found a decrease. The latter fact seems to indicate that the differences in sensitivity towards growth substance between shoots and roots must be ascribed to different wall structure, and not to the protoplasm.

The results of Ruge and Robbins and Jackson, indicating direct action of growth substance on the cell wall without any intermediation of protoplasm, are of great interest, but it would be beyond the scope of this work to go more fully into the matter. In this connection attention must be drawn, however, to the controversy between Fitting (1936) and Went (153) on the principle of stimulation. Fitting considered growth substance as a physiologically stimulating material, which view includes intermediation of protoplasm.

Thimann and Sweeney (15) and Sweeney and Thimann (144) discovered an accelerating effect of it on protoplasmic streaming, and concluded that such is the first visible effect in the chain of reactions leading to elongation. Although this effect occurs immediately after application of growth substance, it is highly probable that it is an accessory result of the action, taking place separately from its action on elongation. This is indicated by the fact that with higher concentrations, retardation of streaming occurs, whereas elongation increases.

According to Ruge (129), changes in viscosity of protoplasm, as first described by Strugger (143), do not occur immediately after application of growth substance, but later than the effect on protoplasmic streaming, so that it is not probable that changes in streaming are due to these changes in viscosity.

Went (154) endeavored to explain the effect of growth substance on protoplasmic streaming as an effect on respiration, the latter in turn influencing the rate of streaming. He assumed that the effect of growth substance is in a thin surface layer of the cell, outside the semi-permeable membrane. As respiration would be increased only in this small region, no differences would be detectable in gross respiration measurements. According to the author of this review, it is also possible that action of the growth substance on protoplasmic streaming takes place without intermediation of respiration and separately from its effect on elongation. A further possibility is that the cell wall influences streaming, so that changes in the wall due to growth substance in their turn affect streaming.

The parallelism between streaming and orientation of molecules, as found in some walls (this will be described in chapter IX with regard to *Phycomyces*), may be considered to denote a relationship of that kind. This hypothesis would also be in accordance with the fact that growth substance probably influences molecular forces in the wall (probably secondary valence forces, compare chapter IX), and it is just these forces which presumably influence protoplasmic streaming. Perhaps electrical charges of the wall which, according to Ramshorn (126), go hand in hand with the rate of elongation (compare chapter IX), are in some way involved in protoplasmic streaming.

Summarizing, it may be stated that the study of protoplasm and its functions and properties as influenced by growth hormone has not contributed very much in revealing the nature of the action of growth substance nor of changes in properties of the cell wall resulting from its action. Study of cell-wall structure, however, has yielded results. The changes of structure were studied in different ways, indirectly by the strain extension relation of the cell wall, to be described in the next chapter, and directly by means of X-ray diffraction patterns and of double refraction in relation with elongation, as will be described in chapter IX.

VIII

THE NATURE OF CHANGES IN ELASTIC PROPERTIES OF THE CELL WALL AND IN DECREASE IN PLASTICITY

In chapter II it has already been mentioned that extensibility and elastic extension of cell walls decrease as a result of inhibited elongation and that both increase as a result of elongation. On the basis of various experimental data two different explanations, which do not exclude each other, have been given concerning these effects:

The Possible Role of the Thickness of the Wall

The first hypothesis (66) was that elastic extensibility is proportional to thickness of the cell wall, *i.e.*, to the amount of cell-wall material present, the modulus of elasticity being constant. Whether a cell wall becomes thinner or thicker was supposed to be dependent on the relation between increase of cell-wall material and rate of elongation. Decreased elongation would result in a thicker wall and hence in less extensibility, while increased elongation

would result in a thinner wall with correspondingly greater extensibility. During normal elongation both processes would counteract each other, which counteraction would result in almost constant elastic extensibility. The only experimental fact supporting this hypothesis was that contrary to what happens at normal temperatures, at 0° or 4° Celsius no decrease of elastic extensibility takes place, if elongation is stopped. Cell-wall formation may also be expected to be stopped at that temperature.

Some data of later authors agree with this hypothesis. Ruge (129), for instance, determining the thickness of walls from their double refraction, found increase of thickness to occur after decapitation. His method is, however, somewhat doubtful perhaps because increased crystallinity of the wall may interfere with the results. Bonner (133), whose results have already been mentioned, made it highly probable, by direct determination of the weight of the wall, that at 0° Celsius cell-wall formation is stopped. On the other hand, he found that at normal temperature the formation closely parallels elongation, although the processes are not necessarily linked together. This is in contradiction to the results of Ruge. Went (152) also accepts the above hypothesis.

The Strain Extension Relationship of Elongating Cell Walls

The phenomenon later (69) appeared to be more complicated in so far that not only the absolute quantity of cell-wall material but also the structure of the wall, perhaps by itself, might control the elastic properties. This followed from studies on the strain extension relationship during elastic stretching of the wall (69). The later data of Gessner (56) pointed in the same direction. In hypocotyls of *Helianthus* it was found that decrease in extensibility of the wall occurred also at 0° to 4° Celsius, at which temperatures formation of cell-wall material is supposed to stop. It is clear, therefore, that decrease in extensibility is not caused solely by formation of cell-wall substance.

In studies of the present author the elastic extension of young elongating cell walls plotted as a function of the strain applied proved not to be represented by a straight line but by a hollow curve which formed a gradually smaller angle with the strain axis (66). The curve begins very steep, finally becoming a straight line with a slightly rising constant slope. Extension of the cell

wall in the turgescent cell corresponds to a point on this final straight part of the curve. In cases of different extensibility only the first steep part of the curve was different, being shorter or longer, the second straight part remaining unchanged, keeping its angle constant with the strain axis in all cases. This suggested that structural properties of the wall were involved, as otherwise (changes of quantity) the entire curve would have changed, the differential over its complete length being increased or decreased. The general shape of the extension curve was attributed to rotation of long micelles, fibrils or molecules of cellulose in the wall, which at first were distributed at random or in transverse direction, but which during extension tended to become parallel with the direction of strain (69, 90). The gradually increasing resistance of the walls against further extension can be explained by this rotation of micelles. The second straight part of the curve would denote that further rotation of micelles is not possible, "structural extension" having reached its limit. Further elastic extension of the wall must then be ascribed to elastic extension of the cellulose fibrils themselves, this corresponding to the final straight part of the curve. A comparison was made with similar phenomena occurring in colloids, and reference was made to Poole (117) who investigated the extension of gels of cellulose acetate [compare also the later investigations on extension curves of colloids by van Iterson (82, 85), Mark (97), Ebbinge (1934) and Bonner (14)]. Rotation of micelles was earlier suggested as an explanation of the great extensibility of fibres by Sonntag (1909) and Steinbrinck (1925).

Direct proof of rotation of cellulose components during extension of the young cell walls of coleoptiles was later given by X-ray investigations of this material in the extended and the contracted state (compare chapter IX). The extensibility of young elongating cell walls is very great, amounting to 20 per cent or more before the breaking point is reached. This can be explained by presuming the presence in the wall of very long fibrils of cellulose which do not adhere together but are able to pass freely by each other. Reference was made in this relation (73) to the researches of Busse (25) who studied the same question in colloids. This author explained the high elastic extensibility of colloids by the presence of long molecules having weak secondary valence forces as a result of hydration: "The absorbed layer insulates these secondary valence forces."

The above explanation of the elastic extension curve of young cell walls was further substantiated by the present author by experiments on the influence of absolute alcohol on extensibility of the wall (72). The shape of the extension curve of the same walls after treatment with absolute alcohol changed in such a way that only the second straight part remained, the straight line passing through the intersection of the two axes and having precisely the same slope as the second part of curve before treatment. This supports the hypothesis that the second part of the curve is due to elastic extension of its cellulose fibrils without rotation and that the first part of the curve in the untreated wall is dependent on the mutual moving and rotation of the fibrils in a hydrated condition, this hydration allowing greater movement, as suggested for colloids by Busse. Dehydration of the wall under the influence of absolute alcohol causes the cellulose fibrils to approach each other and to adhere together so that the corresponding first part of the curve is lost.

The decreased elastic extensibility and the shortening of the first part of the extension curve of young cell walls as a result of inhibited elongation can be explained from the same point of view, by accepting that mutual passing of fibrils has become more difficult, owing to improved coherence of micelles. The cellulose framework thus becomes more stable, and rotation of the fibrils less easy. This explanation is in accordance with X-ray results of the present author which proved crystallinity to be less pronounced in young walls than in old walls and fibrils (72).

Besides rotation of micelles, as described above, interaction of layers in the wall or different extensibility probably also plays a part, especially in epidermal walls (69). This follows from the fact that during contraction of the thick epidermal wall of coleoptiles its inner surface becomes crinkled and folded, while its outer surface remains smooth. These observations of folding were confirmed by Söding (138). The question whether any connection exists between crinkling and other transverse structures (compare Correns, 1892) will be left open. Crinkling of the contracted cell wall denotes compression of the inner layers by contraction of the outer. This effect was explained by presuming that elastic contraction of every layer in the wall is dependent on the degree of rotation that its fibrils have attained, and that these degrees are

different in different layers. On unstretching, the outer layers of greater micellar rotation undergo greater contraction than the inner. The innermost layers, of which the fibrils have not yet undergone rotation, being newly deposited in transverse orientation (compare the theory of van Iterson, to be described in the next chapter), do not contract at all, and are, therefore, compressed into a crinkly state by contraction of the outer layers.

This presumed different orientation of cellulose micelles through the thickness of the epidermal walls also agrees with X-ray results to be described in the following chapter from which orientation of micelles in the direction of the long axis of the epidermal wall can be concluded, micelles of different orientation, however, also being present.

On the foregoing pages, elastic extension of separate cell walls has been dealt with. Regarding plastic extension the following point must be emphasized. During plastic extension of colloidal matter a very intense permanent effect on rotation of micelles generally also occurs. Permanent rotation to that extent can not be expected, however, in walls of turgescient cells, as the coherence of micelles in transverse direction—a cellulose ring being around the wall—impedes such rotation. If, however, the walls of plasmolyzed cells are studied, instead of turgescient cells in which contraction of the wall in transverse direction is possible, it appears likely that permanent rotation of micelles under artificial extension also takes place, if only the wall plasticity is sufficient.

Summarizing, it may be concluded that elastic extensibility and extension of young cell walls must be explained on the basis of rotation of micelles, combined with superimposed interaction of wall layers of different micellar orientation and extensibility. During plastic extension of the walls of turgescient cells permanent rotation of micelles is impeded by the transverse coherence of micelles round the total cell, so that permanent orientation of micelles during elongation will be less than in colloids or separate cell walls.

Decrease in elastic extensibility of the wall in the course of time can be explained as an improved coherence of cellulose fibrils (increased crystallinity, decreased hydration, or relaxation of rotated micelles). Increase of elastic extensibility as a result of elongation can be explained by inhibited coherence of cellulose micelles during plastic stretching, the micelles continuously sliding along

each other. In this way the micelles become or remain more independent of each other, so that greater motion, which results in greater elastic extensibility, is possible. There may be interaction of wall layers of different micellar rotation. It is not impossible, but rather improbable, that the amount of cell-wall substance (cellulose) also influences extensibility.

IX

THE MOLECULAR AND MICRO-CRYSTALLINE STRUCTURE OF THE CELL WALL IN RELATION TO ELONGATION

X-ray Investigations on Young Elongating Cellulose Walls

Although many X-ray investigations had been carried out on cellulose of plant cell walls, only a few kinds of fibres had been studied (Sponsler, Meyer and Mark, Herzog, Polanyi).⁶ Young elongating walls of cotton hairs were considered by Clark (39), and of the unicellular alga *Valonia* by Sponsler (140), but the relation between molecular structure of the wall and elongation had not been considered. Therefore, X-ray diffraction patterns of young elongating walls of coleoptiles and other material were investigated by the present writer for further analysis of the action of growth substance on the plasticity of the cell wall (70, 72). The materials investigated were epidermal strips of coleoptiles, the epidermis of the leaves of *Muscari* and of the hypocotyls of *Lupinus*, as well as the parenchyma of hypocotyls of *Lupinus* and of the stem of *Helianthus*. The epidermis of coleoptiles was investigated after drying in the extended and in the contracted state, as well as in the wet state, as in living cells.

Typical two-point diagrams were obtained, especially of older coleoptiles, after drying in the extended state. All typical interferences of cellulose were present, most of them on the equator. The calculated identity periods were $A_1 = 6.31$ Å (corresponding to the planes of Miller indices 101 of cellulose); $A_2 = 6.10$ Å (planes 10 $\bar{1}$ of cellulose); $A_4 = 3.95$ Å (planes 002 of cellulose); and an outer very sharp but faint interference ring = 2.56 Å (corresponding to the fiber periods of cellulose planes 040, 4d being 10.24–10.28 Å).

⁶ For a general survey of the structure of the plant cell-wall the reader is referred to Vol. 1, p. 52, of this periodical.

Interferences A_1 and A_2 were more vague in the young cell walls than in the cellulose fibers. This fact was interpreted as denoting a less developed crystalline state of cellulose. Interference A_3 was absent, and interference A_4 , differing from that of fibres, consisted of three distinct zones. The middle zone corresponded only to the typical interference 002 of cellulose.

The typical two-point diagram, from preparations dried in the extended state, indicated an orientation of cellulose crystallites perpendicular to the equatorial plane (compare Polanyi, 1921; and Polanyi and Weissenberg, 1922), *i.e.*, parallel to the long axis of the epidermal walls. It can be concluded, therefore, that the direction of cellulose molecules in the completely extended young epidermal wall is parallel to the long axis of the cell. A less typical two-point diagram was obtained if the same epidermal wall was previously dried in the contracted state, the interference 002 of cellulose being represented by a complete ring with two arcs of higher intensity instead of the two interference points on the equator in the former case (compare figures 1 and 4 of (72)). The interferences A_1 and A_2 were also represented by arcs. This indicates more random orientation of crystallites with less orientation in the lengthwise direction. It is presumable, therefore, that elastic extension is a cause of increased orientation of micelles in the lengthwise direction. These data are in complete agreement with the conclusions derived from study of the strain extension curves described in the foregoing chapter, and the conclusion there that the rotation of cellulose molecules and crystallites is a result of elastic extension, is proved by these X-ray results.

Besides these typical cellulose interferences there were others in the young cell walls. Interference A_4 could be separated into three different zones. The middle zone, A_{4a} , corresponding to an identity period of 3.92 Å (ranging from 3.83 to 4.08 Å), fully corresponds to interference A_4 of the plane 002 of cellulose. On the inside of it, an additional interference, A_{4b} , was present corresponding to an identity period of 4.18 Å (ranging from 4.30–4.08 Å). On the margin between these two there was a very intense and clearly defined interference line, A_{4c} , corresponding to an identity period of 4.08 Å. On the outside of the cellulose interference A_{4a} , an additional interference, A_{4d} , corresponding to an identity period of 3.75 Å (ranging from 3.83 to 3.62 Å) was finally found. No

definite conclusions were made with regard to the meeting of these additional interferences (A_{4a} , A_{4b} , A_{4d}). It was noted only that interference A_{4a} completely agreed with the same interference ranging over an exactly similar area, obtained with the same camera, from dried pectin. It further appeared that these additional interferences, A_{4a} and A_{4d} , were represented by points on the equator when the material was examined in the stretched condition. The interference A_{4b} , however, was always represented by a clearly defined ring. If the interference A_{4a} is to be ascribed to pectin, it must be concluded that the molecules of this substance also have definite orientation parallel to the long axis.

The possibility was further mentioned that the A_{4a} interference might be due to cellulose in a less perfect crystalline state, as was also indicated by the vagueness of the interferences A_1 and A_2 . Clark (39), in a study of cotton hairs at various ages, arrived at conclusions of a similar kind, finding displacement of interferences of cellulose during aging, the identity period gradually decreasing from 4.47 to 3.90 Å. He ascribed this to improving crystallization of the cellulose molecules during aging (72).

Living epidermal cell walls of coleoptiles in the wet state showed interference A_{4a} only as a faint ring on the inner side of the broad diffused interference of water, this interference of water becoming more clearly defined on the inner edge. By slight drying, this inner interference became still more clearly defined. It was suggested from these data that in young walls of elongating cells the cellulose molecules have less or perhaps no crystalline structure, perhaps being present only in a mesomorphic or paracrystalline state; Friedel (1928) and Rinne (127) described such a mesomorphic state in colloids.

In this relation it was emphasized (72) that double refraction of cell walls should be found also if long molecules of definite orientation, but not in a crystalline state, are in the wall, and reference was made to Smiles and Herzog (1914) and to Katz (1928).

The hypothesis of a less crystalline structure which in the course of time becomes more complete would be in good agreement with the explanation given above of the plastic and elastic extensibility in the course of time when elongation is checked.

On the other hand, the question whether the additional interferences must be ascribed to other substances present was studied more

closely. Besides interference A_{4a} , which was originally compared to that of pectin, and interferences A_{4b} and A_{4d} , Kolkmeier and Heyn (90) found still other interference lines on the outside of A_4 ; most of these corresponded with those of ice, and by analogy the possibility was suggested that most of the additional interferences are due to the hydration film surrounding the cellulose micelles (compare also Kolkmeier and Favajee (91) who ascribed most of the powder lines of the starch diagram likewise to bound water).

Later, Hess, Trogus and Wergin (63) continued these investigations. They studied cotton fibres at various ages, as well as epidermal cell-walls of *Avena* coleoptiles. They fully confirmed the results obtained with coleoptiles described above, and arrived at still further conclusions. The identity periods of additional interferences, as found by them in both cotton hairs and coleoptiles, were 4.20 and 3.73 Åu, which values agree exactly with those for the interferences A_{4a} and A_{4d} of the cell walls of coleoptiles. Furthermore, they found the cellulose interferences more intense in the epidermis, and the additional interferences more intense in the parenchyma tissue of coleoptiles. They explained the displacement of interferences during aging, described by Clark, by superposition of interferences of cellulose and the different intensities of these two interferences during aging of the wall. It is inconceivable, however, that such superposition plays a part in the diagrams of coleoptiles described above. Hess, Trogus and Wergin suggested the presence of a special substance, called by them "primary substance," in the young cell walls, giving rise to the two additional interferences. They, furthermore, agree with the conclusion that in elongating young cell walls cellulose is not present in a crystalline condition. In another study, Gundermann, Wergin and Hess (58) investigated the additional interferences more closely. They compared them with the exactly similar interferences of special kinds of wax, e.g., of *Copernicea cerifera*. By analogy they concluded that the same substance must be present in the young cell walls, causing the two additional interferences. This suggestion was substantiated by the fact that from the young walls a substance could be dissolved with benzene, giving the same interferences. According to these authors, this primary substance would differ from the cuticular substance.

The conclusion that this primary substance must of necessity

play a part in elongation is not at all substantiated by experimental data except by the circumstance that it occurs in young walls only; on the other hand, the possibility is not completely excluded of its having a definite role in elongation.

Summarizing, it may be concluded that the molecular structure of young cell walls is different from that of older walls and fibers. Crystalline structure of cellulose molecules is either wholly absent from young walls, or poorly developed, the molecules being present in either an amorphous or a paracrystalline or mesomorphic state. Moreover, other substances than cellulose may participate in the X-ray diagram of the young cell wall. These are perhaps primary substances, as supposed by Hess and co-workers; or they may possibly be pectin or bound water. In the dried condition, crystalline cellulose micelles are certainly present in the young wall, and from their interferences in various cases a rotation of cellulose micelles or macromolecules during elastic extension is indicated.

The Spiral Growth of Phycomyces

The spore-bearing cell of *Phycomyces* rotates round its long axis during elongation (Burgeff, 1915). Oort (114) was the first to make a special study of this phenomenon, but was unable to furnish an explanation. Oort and Roelofsen (115), referring to the old hypothesis of Dippel on the relation between protoplasmic streaming and orientation of new particles deposited in the cell wall, were unsuccessful in correlating protoplasmic streaming and spiral growth. No oblique direction of protoplasmic streaming was found in the zone of elongation; this was confirmed later by Pop (119)ⁿ. But oblique streaming was found in older zones where no more elongation took place. The streaming was parallel with certain structures of the secondary cell wall, the presence of these structures being concluded from optical data on the wall.

A study of the double refraction of the wall revealed that it consisted of three different layers. The middle and main layer showed positive birefringence, the long axis of the index ellipsoid of double refraction forming a slight angle only with the long axis of the wall. The long axis of the index ellipsoid of a second, thin layer outside the main layer is almost perpendicular to the long axis of the organ. The birefringences of these two layers compensate each other at a distance of about 2 mm. from the sporogonium, the zone

of elongation lying within these 2 mm. Below this zone the birefringence of the middle layer predominates, whereas above it the birefringence of the outer layer predominates. That the middle layer is even completely absent from the zone of elongation, as concluded by Oort and Roelofsen, is not proved by the optical data. The experimental data can be explained also by a less developed crystalline structure of this layer in the growth zone. In the following it will be proved that in the zone of elongation this layer has less crystallinity. In the older area below the zone of elongation Oort and Roelofsen found protoplasmic streaming and a stripping of the cell wall parallel to the long axis of the index ellipsoid of the middle layer.

With regard to the third layer inside the main layer, it is sufficient to mention that it was very thin and had irregular structure.

The authors ascribed the birefringence of the different layers to the presence of oriented chitin crystallites. The birefringence of chitin itself is very faint, so that "shape" birefringence must cause the double refraction; this was substantiated by the fact that the birefringence of the wall was largely increased by dyeing. These suggestions are taken for granted, but no conclusion is possible as to the shape and orientation of the supposed crystallites and molecules of chitin in the wall.

In 1935 the present author (75, 76) investigated the molecular structure of the wall of this spore-bearing cell of *Phycomyces* by means of X-ray diffraction patterns. It was possible to determine exactly the place of the chitin molecules in the wall and dimensions and structure of the crystallographic cell of chitin, which up till then had not yet been established. Determination of orientation of molecules in cell walls had been previously accomplished only in the cellulose walls of *Valonia*, studied by Sponsler (140) and later by Preston and Astbury (124).

The chitin molecules which form the frame of the wall in *Phycomyces* can be conceived as consisting of ordinary cellulose chains, built up of linked glucose residue rings with short proteid side-chains alternately on each side, one side-chain corresponding to one glucose residue ring, together with it forming one glucosamin residue.

The chitin proved to be situated as follows in the hollow cylinder formed by the cell wall. The axes of the proteid side chains lay

exactly in radial direction, perpendicular to the surface on the wall, horizontally separated from one another by 4.6 Å (corresponding to the identity period of proteids), and vertically separated from one another by 10.15 Å, nearly corresponding to the fibre period of cellulose). It was deduced from these data and the asymmetrical structure of the chitin molecule that the cellulose main chain of the chitin molecule is nearly parallel to the long axis of the organ, forming an angle of 27° or $13\frac{1}{2}^\circ$ with this axis.⁷ With regard to the other directions, the chain must be in such a position in the older wall that the planes of the glucose anhydride rings (of the glucosamin residues) are perpendicular to its surface.

This conclusion on the position of the chitin molecules in the wall can be brought in accordance with the results on double refraction obtained by Oort and Roelofsen, if it is assumed that the long axis of the index ellipsoid is parallel to the lengthwise direction of the chitin molecules, and that the X-ray diffraction pattern is due principally to the thick main layer of the wall as described by these same authors. Accepting these assumptions, it may be concluded that the orientation of chitin molecules in the thin outer layer is perpendicular to the orientation of the molecules in the main layer. The outer layer, therefore, can be described as having "tube" structure, for it consists of long chitin chains or micelles encircling the organ, these chains being almost perpendicular to the long axis of the organ. In the same way the main layer can be described as having "fibre" structure.

For the explanation of spiral growth it was also of interest to compare the structure of the cell wall in the zone of elongation with that of it in older parts of the organ. X-ray diagrams showed striking differences. Of the elongating zone, the diagram was much more diffused, indicating a considerably less pronounced crystalline state. The interferences of the fibre period were, however, clearly defined, and indicated an orientation of molecules in the lengthwise direction of the organ. A diagram of the young growing stem of *Pholiota* did not show even these interferences, but had only a broad diffused ring, indicating the absence of any definite crystal lattice.

⁷ The long axis of the chitin molecule forms an angle of 27° with the long axis of the organ and with the vertical planes of spacing of the proteid side chains, if all other proteid chains are accepted as lying in one vertical plane above each other. This angle must be $13\frac{1}{2}^\circ$ if every second side chain lies vertical above the one beneath, and if the intermediate one lies half way between in a horizontal position, as was originally assumed.

It is highly probable from these data that the molecules of the main layer of the cell wall in the elongating zone of *Phycomyces* are oriented in the lengthwise direction but do not yet form a complete crystal lattice.

As a perfect crystalline state was found in the older parts of the wall, behind the zone of elongation, it must be concluded that a transition of molecules takes place from the non-crystalline into the crystalline state in the direction from the younger to the older parts of the wall. This conclusion is in complete accordance with the general theory that crystallinity increases with aging of the wall (compare chapter VIII).

Although these new data on the molecular structure of the wall will be of great importance for the explanation of spiral growth, a complete explanation could not yet be given (compare 78) and different possibilities and theories exist at the present time which will next be summarized.

One hypothesis (76) was that during elongation plastic stretching of the wall occurs by sliding of chitin molecules along planes of greatest weakness in the crystallites, these weakest planes being parallel with the glucose anhydride rings. Since these planes form an angle of $13\frac{1}{2}^\circ$ or 27° with the long axis, an angle of spiral growth of the same extent must result.

Another possibility (78) is that in the main cell-wall layer of the elongating zone the chitin molecules are originally deposited in the direction of the long axis, parallel to the direction of protoplasmic streaming. At that moment the molecules have not yet complete crystalline configuration, as can be seen from the X-ray diagram. But very soon they arrange themselves in the denser crystal lattice as in the older zones. This transition to the crystalline state must be accompanied by inclination of the molecules into an oblique position of 27° with the long axis of the organ, for otherwise the oblique orientation of the chitin chains in the crystal lattice in the older wall would not be realized. This inclining of molecules in the circular wall of the elongating zone must go hand in hand with rotation of the unicellular organ; combination of the rotating forces and of the extending forces of elongation then results in spiral growth. As inclining of the chitin molecules is probably caused by forces of crystallization (secondary valence forces; presumably also attraction between CO and N groupings in the crystal plays an important

part), the forces causing spiral growth must be identical with those of crystallization.

Castle (26-32) made some interesting observations on the spiral growth of *Phycomyces*. He detected the important fact that temperature influences the rate of rotation much more than the rate of elongation, so that the steepness of the average inclination of the spiral (with the long axis) is greater at higher temperature. Higher temperature—between 27° and 28° Celsius—abolish rotational growth altogether or rarely produce rotation in opposite direction, elongation continuing. Castle concludes that the crystal structure of the cell wall can not be the cause of spiral growth, for in that case temperature would influence rotation and elongation in a similar way.

If only the fact that temperature has greater effect on formation of new cell-wall substances than on elongation is considered,⁸ the different influence of temperature on rotation and elongation could be interpreted, according to the author of this review, just as well on the basis of the explanation of spiral growth by crystal structure. Decrease in deposition of new chitin molecules in vertical direction in the middle layer of the wall must result in decrease of the amount of inclinable molecules. As a result the forces of crystallization and, therefore, the angle of spiraling will be reduced (compare 78). Also the absence of rotation of the spore-bearing cell before formation of the spore mass, as described by Castle, can be interpreted in a similar way by assuming that at that period chitin molecules are not yet deposited in vertical direction in the elongating zone, only the primary wall, of which the molecules have transverse orientation, being present.

Anyhow, the experimental data of Castle are not necessarily in contradiction to the explanation given here of spiral growth, according to which crystal structure or crystal forces are the direct cause.

Endeavoring to explain the spiral growth of *Phycomyces*, Castle, on the other hand, suggested that it is due to interaction between turgor and elastic forces in the membrane. This suggestion he based on observations on a special very illustrative model, consisting of parallel vertical dowels fixed at their bases. A narrow compression ring pushed over the dowels causes them to lean slightly in against

⁸ The temperature coefficient of rotation was found to be 2.5 by Castle, whereas in chapter II a coefficient of 1.2 was given for a special case of plastic stretching of the cell-wall.

the spool at the top. Spiral twisting occurs if the ring is pushed down, and the dowels then produce a complex curvature with an inflection point. This model occurs with nature in so far that the elongating zone of the organ, tapering towards the top, also shows a similar kind of sigmoid curve of its cell wall. Furthermore, Castle finds that in cells of different types of curvature of the cell wall in the elongating zone the angle of spiraling also tends to be different. By these experiments a causal relation between curvature of the cell wall and rotation is made very plausible, but it would be too far fetched to presume from this analogy that spiral growth must necessarily be explained by a twisting of elastic elements of the cell wall, resolving bending stresses imposed on them by turgor pressure. The compressing ring in the model can not be compared with any force in the organ, a direct comparison with turgor pressure being hardly possible. Perhaps a combination of these ideas with the data on the molecular structure of the wall will prove useful.

Preston (122) has later shown from a simple geometrical treatment of the problem that it is possible to obtain right and left hand rotation merely by presupposing a spiral arrangement of chitin molecules in the primary cell wall. Disputing Castle, he ascribed spiral growth to spiral structure of the cell wall.

Investigations on the Micro-Crystalline Structure of the Cell Wall

In chapter VII and in the beginning of this chapter the strain extension curve of cell walls was explained by rotation of micelles and simultaneous interaction of different layers, a comparison being made with the extension curves of gels. This explanation was proved by direct X-ray observations on extended young cell walls. In addition, Bonner (14) later studied the double refraction of these walls during elongation, and arrived at certain conclusions regarding the orientation of their micelles. On the one hand, he was able to verify the conclusions (VIII) on rotation of micelles during artificial elastic extension by using these methods, but, on the other, he was unable to find this rotation during plastic extension of the wall and normal elongation of the turgescient cell.

Bonner studied coleoptiles after removing the epidermis. Double refraction, as determined from the path of difference, proved to be proportional to the artificial extension applied in the parenchyma walls investigated, as well as in cellophane strips, the double refrac-

tion changing from optical negative into positive. This is what must be expected if in the cell wall rotation of cellulose micelles takes place from the original completely transverse orientation into an orientation parallel with the direction of the extending force. The epidermal wall was optically positive from the beginning, but here also double refraction increased during extension, also indicating increased orientation in the direction of the long axis of molecules or micelles already oriented in the lengthwise direction.

These results are in full agreement with the explanation of the elastic extension curve given in chapter VIII and the first part of this chapter. Bonner left out of consideration only the interaction of layers of different micellar orientation in the wall, as indicated by the compression of inner layers on contraction.

As already mentioned, Bonner detected the important fact that, on the other hand, a similar rotation of micelles does not occur during normal elongation. He found that the transverse orientation of cellulose micelles in the parenchyma cell walls remained stationary in coleoptiles of successive ages, and he therefore concluded that during elongation rotation of micelles does not occur. Orientation of micelles also did not occur in coleoptiles elongating in a solution of growth substance, regardless of whether this occurred at normal temperature or at 4° Celsius. This differs from what happens in the course of time during extension of cellophane strips where permanent reorientation of micelles in the direction of the strain is the result of plastic stretching.

According to Bonner, the absence of any permanent reorientation of micelles in the wall during normal elongation can be explained only by presuming active interposition of new micelles between those already present, *i.e.*, active growth of the wall compensating the effect of reorientation. Therefore, according to Bonner, active growth of the cell wall, in addition to plastic stretching, plays a part in causing elongation.

It is difficult to accept this explanation, however, in accounting for the absence of reorientation during elongation at 4° Celsius, as formation and interposition of new micelles does not take place at this low temperature. Bonner assumes a transformation of substances already in the wall.

Preston (123) repeated the investigation on double refraction of the parenchyma cell walls of coleoptiles during elongation. Instead

of studying the complete tissue which after removal of the epidermis consists of 5 double cell walls (the coleoptile sheath consists of 5 cell layers; each wall consists of the 2 single adjacent walls of neighboring cells), he examined single walls of the same tissue, prepared for examination by a special technique. In various points his results differ from those of Bonner. In the first place, the extinction position, if studied in single walls, was always inclined at a considerable angle to the transverse direction. The different results of Bonner were explained by Preston as being due to interference of the opposite extinction directions of the two adjacent walls of neighboring cells; if the angle of the inclination is below 45° a transverse major extinction position of the double wall must result; if the angle of inclination is above 45° the direction of the major extinction position of the double wall must be parallel with the long axis.

Contrary to Bonner's observations, Preston found that in cells of increasing length and age, *e.g.*, during elongation, the birefringence of the double wall changed from negative into positive. These data indicate a rotation of micelles into the lengthwise direction during elongation, just as it occurs during plastic stretching of colloids, but probably to a lesser extent.

Exactly as described by Preston (123), Frey-Wyssling (51) also observed reorientation of micelles during elongation of the rapidly elongating filaments of grasses. From these observations it was concluded by the latter that in this case elongation occurs by mere plastic stretching. This conclusion is in complete accordance with data on plastic stretching in the same material described by Schoch Bodmer (134). In other cases, however, where no reorientation of micelles was detected, according to Frey-Wyssling, active growth of the wall would occur simultaneously with plastic stretching, both processes being direct causes of elongation.

The lesser rotation in turgescient elongating walls in the sense of Bonner and Frey can be explained, according to the author of this review, by two facts. The first, already mentioned in chapter VIII, is that the coherence of cellulose micelles in transverse direction around the turgescient cell resists rotation of the micelles during plastic stretching, a contraction of the wall in transverse direction being impossible in turgescient cells. Plastic stretching of young cell walls during elongation, therefore, must be considered to be much more a passing along each other of rings of cellulose micelles

encircling the wall, the rings slightly changing into ellipsoids during elongation. This state of affairs is rather different from what occurs if strips of colloids are extended, in which case contraction of the substance in transverse direction is easily possible, and indeed occurs (compare Iterson and Buyn, 85).

The above assumed coherence of micelles in the direction of their long axes is in accordance with modern ideas on micellar structure, as suggested by Abitz and Gerngross, and in a modified way by Iterson and Buyn (85) for colloids, and by Meyer (104), Iterson (33) and Frey-Wyssling (50) for cellulose walls. All these authors conclude that one molecule participates in the building up of more than one micelle.⁹

The second fact which probably also plays a part in decreasing the effect of plastic reorientation of micelles during elongation, is that during this process new particles are deposited in the original orientation.¹⁰ According to the theory of van Iterson—to be described in the following—the orientation of new particles in the young cell wall always takes place with the long axis of the new particles perpendicular to the direction of elongation. The effect of a reorientation of micelles in the wall during elongation will be obscured by this deposition of new particles in transverse direction, the double refraction of the new particles interfering with that of the older already rotated particles.

If these suggestions are taken for granted, the conclusion of Frey-Wyssling and Bonner on the role of active cell-wall growth becomes superfluous in explaining the absence of rotation. The later explanation by Frey-Wyssling and Schoch-Bodmer (52), in attributing the changes of negative into positive birefringence in the filaments of grasses to stress birefringence, also becomes unnecessary.

Regarding the orientation of cellulose micelles in transverse direction in the primary wall, van Iterson (85, 84, 85) formulated a more general theory in which he stated that orientation of micelles in the primary wall is always perpendicular to the direction of elongation ("Tube structure" of the primary wall). This theory was substantiated by van Iterson with data on the orientation of micelles

⁹ See, however, Kerr (*Protoplasma* 27: 221. 1937) and Wergin (*Naturwis.* 26: 613, 1937) who described very regular particles of definite size (0.2μ) in the secondary wall. The structure of the secondary wall is, therefore, probably different from that of the primary wall as described by the above-mentioned authors.

¹⁰ But without being a direct cause of elongation in the sense of Bonner.

in transverse direction in the seta of *Pellia* (83) and in filament hairs of *Tradescantia* (84), and recently by Ziegenspeck (156) who noted that in organs capable of movement the direction of greatest movement is always perpendicular to the long axis of the cellulose micelles in the walls.

The author of this review goes still further and suggests that the later form of a cell is highly dependent on the original direction of micelles in its young wall.

The theory of van Iterson is in good agreement with the theory of elongation by plastic stretching, for in plastic stretching the direction of greatest extensibility is also perpendicular to the long axis of micelles. (Compare data on gels, and the recent data of Ziegenspeck on cell walls.)

Summarizing, from the results obtained on the micro-crystalline structure of cell walls it can be concluded that the data are in full agreement with the conclusions derived from the X-ray data described before. The smaller effect in rotation, or even the complete absence of rotation of micelles during elongation, can be explained without assuming active growth of the wall besides plastic stretching as a cause of elongation. The theory that surface enlargement of the wall takes place perpendicular to the micelles in the primary wall appears to be very promising.

X

ON THE NATURE OF INCREASE IN PLASTICITY OF THE CELL WALL

Although many of the investigations described in chapters VI-IX were designed to yield further analysis of the action of growth substance on the wall, nothing is yet definitely known regarding the way in which the increased plasticity is thereby brought about. The original hypothesis of the present author was that growth substance influences the hydration of particular cell-wall components (66, 214). It is not that the hydration of the entire wall or the total amount of water is increased, but that transition of bound water into free water, and inversely, probably takes place. Especially hydration of the cellulose molecules of micelles was considered later; X-ray investigations pointed to less complete crystalline structure in the younger elongating wall, which fact was interpreted to mean greater hydration of the cellulose molecules. Furthermore, reference was made to the investigations of Busse on gels, showing

that the secondary valence forces of colloidal particles are insulated by the hydration film of water "so that a greater extensibility results." Kolkmeier and Heyn (90), on the basis of X-ray investigations, considered it plausible that the bound water in young cellulose cell walls has regular crystalline structure, similar to the structure of ice.

Bonner and Heyn (1935), expecting electrical charges of cell-wall particles to play a part, investigated the cataphoresis of wall particles, but could not find any influence of growth substance. On the other hand, Ramshorn (126) found close parallelism between the rate of elongation in different zones of the elongating region and their electrical charges, as determined in the living plant. This electrical potential could probably be a result instead of a cause of plastic stretching which had already taken place in the walls.

Frey-Wyssling (50) and Bonner (14), accepting both plastic stretching and intussusception as causes of elongation, suggested that the junctions of the cellulose network in the wall are directly or indirectly loosened by growth substance. This would mean that the secondary valence forces are weakened by growth substance, as already suggested above in reference to Busse. Experimental support for this attractive hypothesis is lacking, however. It would be in full accordance with the theory of elongation given in this review. With regard to the way in which coherence of cellulose micelles at these function points can be weakened, Ruge (132) published some valuable experimental data which, it is to be regretted, are based on analogy only. Studying the swelling of organic materials under the influence of growth substance, he found that the swelling of pectic materials was greatly increased. This made Ruge conclude that probably hydration of pectic substances is increased in the wall by the growth substance. According to him, growth substance acts directly on the wall, and in this way, by a swelling of the interposed pectic substances, the cellulose molecules of the walls become separated from each other, so that decreased mutual attraction results. Aging of cell walls and decreased reactivity to growth substance, according to Ruge (131), are attributable to transformation of one kind of pectic substance into another, or of lessened ability to absorb water.

In this relation the investigations of Wuhrmann (155) must be mentioned, too. He noted that all neutral salts decrease both plas-

ticity of the cell wall and rate of elongation. Von Dellinghausen (1933) found in a similar way that the swelling of agar-agar, also a cell-wall component, is decreased by all neutral salts.

It is also of interest to note that, according to Haagen Smit (1937), many other substances besides auxin can increase elongation, if present in parts of the cell wall capable of elongation. Inactivity of these substances in normal elongation is due only to the fact that they are not subject to polar transport in the organ.

It must finally be emphasized that the new theory on elongation given in chapter II and further discussed in the next pages of this review, does not imply that the presence of growth substance is a necessary condition for elongation. This question is left open. Growth substance was used only as means of analyzing the process. Elongation might be possible in the absence of growth substance if the cell wall has sufficient plasticity (compare the data of Overbeck (116) on the seta of *Pellia* and some recent data of Avery and La Rue.) (Bot. Gaz. 100: 186. 1938.)

In final conclusion it may be stated that from the foregoing critical survey of all available experimental data the new theory on elongation, as expounded in this paper, appears to be fully confirmed.

* * * * *

Since preparation of the foregoing review, the following contributions on the subject have appeared:

Experiments on hypocotyls of *Helianthus* (158) have shown that the primary effect of growth hormone is greater swelling capacity of the cell wall. This first effect must directly result in elongation of the wall (plastic extension). After the first phase, new cell-wall material is deposited in the meshes of the cellulose frame. The next effect of the growth hormone is to weaken the junctions of the cellulose frame. These ideas are in full agreement with the theory of the present author and explain the effect of the growth hormone on plasticity as independently influencing the junctions of the cellulose net and the swelling capacity of the cell wall.

A new element is introduced by these authors in assuming that the newly deposited cell-wall material also attracts water and thereby causes further swelling.

Ziegenspeck (159, 160, 161) performed investigations proving the theory that cell walls undergo plastic and elastic extension most easily in the direction perpendicular to the axes of the micelles.

Martens (162) continued his investigations on the plasticity of staminal hairs on stamens in *Tradescantia*, and again showed the extreme plasticity (up to 100%) of these walls after the cuticle is removed.

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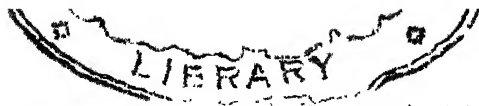
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POLYEMBRYONY

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INTRODUCTION

Advancement of our knowledge of polyembryony¹ has been very slow in comparison with the progress made in other botanical fields. This slow development may be attributed to the fact that, except in relatively few genera, polyembryony is very limited in occurrence and only occasionally observed. This sporadical occurrence has greatly handicapped studies of polyembryony, and has led to the belief that adventitious embryos are malformations. Despite the slow rate at which knowledge of the subject has progressed, the scattered literature in this field clearly shows that significant contributions have been made.

Polyembryony was first reported by Leeuwenhoek in 1719, when he found orange seeds each containing two embryos. During the next century and a half, cases of polyembryony were reported and listed. In 1878 Strasburger demonstrated the formation of plural embryos in several genera, and there followed a period in which numerous embryological studies were made. As a result the main modes by which adventitious embryos are formed were established, and several theories as to causes of polyembryony were advanced. In 1901 Ernst summarized the literature dealing with polyembryony and classified the various means by which adventitious embryos may be derived. Following Ernst's work, it soon became apparent that polyembryony is not abnormal, and studies turned from the formation of plural embryos to the resulting seedlings. Although at first very little genetics was involved in these studies, modern methods and principles of cytogenetics were soon applied. Such analyses during the past decade have been of great value and have shown that in many genera polyembryony plays an

¹For general references on polyembryony the reader is referred to Ernst (31), Schnarf (88), Frost (36) and Buchholz (11).

important role in practical breeding work and in both the origin and the perpetuation of new forms. With this knowledge, plural embryos can no longer be considered as monstrosities which lack interest and importance in botanical studies and agriculture.

TYPES OF POLYEMBRYONY

In recent years polyembryony has been reported in numerous species, genera and families of both the gymnosperms and angiosperms. It is not limited to any type of seed nor any type of embryo sac development. In numerous species it has been possible to trace rather fully the formation of the adventitious embryos. In other species, however, polyembryony occurs so rarely that only partial tracing of the process has been possible, and at times the origin of adventitious embryos has been judged chiefly by their location within the nucellus or embryo sac. Further evidence as to the possible origin of multiple embryos is obtained from the genetical and cytological composition of the polyembryonic seedlings.

Sporophytic Polyembryony

Recent literature reports adventitious embryos derived by sporophytic budding from the:

- 1) Nucellus: Rutaceae—*Citrus* (32, 33, 34, 78, 91, 99, 102, 108, 110), *Poncirus* (34, 68), *Fortunella* (34, 68), *Zanthoxylum* (18), *Murraya* (18), *Aegle* (18); Anacardiaceae—*Mangifera* (4, 48, 50, 52, 107); Rosaceae—*Potentilla* (38); Myrtaceae—*Eugenia* (80); Betulaceae—*Alnus* (111); Malpighiaceae—*Hiptage* (96).
- 2) Integument: Myrtaceae—*Eugenia* (80); Rosaceae—*Potentilla* (92).

The production of sporophytic embryos begins soon after flowering in enlarged cells of the integument or nucellus. These cells divide, grow and project into the embryo sac, then develop into one or several embryos. The metamorphic embryos thus formed either compete with or replace the gametic embryo.

Opinions differ as to whether or not the formation of metamorphic embryos is stimulated by pollination and fertilization. Wright (112) found that such embryos develop without pollination. However, Frost (34), Wong (110), Gentscheff (38) and others found that parthenocarpic fruits may develop without polli-

nation but that seed formation depends on pollination. Toxopeus (101) and Juliano (50) observed that metromorphic embryos develop after amphimixis, while Pijl (80) concluded that such embryos originate autonomously but cease development if fertilization is not effected. In reviewing the literature, Gustafson (39) concluded that some substance from the pollen tube stimulated metromorphic embryo development. Laibach (64), Laibach and Masehmann (65) and others have shown that pollen is rich in growth-promoting hormones.

Since metromorphic embryos are pseudogamic in derivation, the seedlings arising from them are, with few exceptions, identical with each other and with the mother. Although in some species gametic seedlings can not be distinguished from metromorphic seedlings (50), in others they are fairly easily separated. In the latter case the differentiation is not due to any abnormality in the formation of the gametic seedlings, but to the highly heterozygous condition of the species (34). The few metromorphic seedlings which differ from the mother are usually tetraploids (34, 68) or albinos (34). Frost (34) concludes that these albinos are due to chimera conditions in the mother, which the mother fails to exhibit, either because they are too small or because they are covered by green tissues. The fact that diploid and tetraploid metromorphic seedlings are formed side by side led Frost to believe that tetraploids also arise from maternal chimeras, or possibly by chromosome doubling shortly after formation.

Cleavage Polyembryony

(Cleavage polyembryony is accomplished by the separation of the zygote or young embryo into two or more units, each of which develops into a separate embryo.) In the Coniferales and *Gnetum* a proembryo containing embryo initials or a cell which gives rise to them, is formed shortly after amphimixis. These initials give rise to independent embryos that are usually separated from each other. (In some genera cleavage embryos are also formed from proliferations of the proembryo (28, 11) or from differentiated cells, such as the prosuspensor cells (13). Cleavage polyembryony has recently been reported in *Sequoia* (16), *Cupressus* (27), *Cryptomeria* (14), *Sciadopitys* (12), *Juniperus* (72, 21), *Chamaecyparis* (13) and *Saxegothaea* (28).

Although the term cleavage polyembryony is usually restricted to the above described process in the gymnosperms, it is equally applicable to this process in angiosperms. It is, however, comparatively rare in the angiosperms, having been observed in only a few genera, mainly *Loranthus* (Loranthaceae), *Erythronium* and *Tulipa* (Liliaceae), *Limncharis* (Orchidaceae) (31), and *Lobelia* (Lobeliaceae) (24). In these cases plural embryos generally arise from the same portion of the proembryo, as in the gymnosperms, but they are derived from proliferations rather than from definite embryo initials. Recently reported cases of cleavage polyembryony in the angiosperms are based upon polyembryonate seedlings.

(Since the embryos in cleavage polyembryony are monozygotic in origin, the resulting seedlings are identical. This view is supported in *Linum* (Linaceae) (55) by multiple seedlings that were genetically identical as evidenced by both flower color and multiple factor characters. In the Leguminosae, Skovsted (90) reported twins in *Trifolium*, both members having a chromosome fragment, and in *Medicago* twins with both plants having an extra chromosome. Cameron² reported triplet seedlings in monosomic *Nicotiana tabacum* \times normal *N. tabacum* (Solanaceae) which were all monosomic and identical in respect to all characters involved in the hybrid. In the Gramineae chlorophyll-deficient twins in *Holcus* (56) and identical twins and triplets in *Oryza* (83) were assumed to be of monozygotic origin. Identical twins in the gametic progeny of *Citrus* (34, 105) were attributed to embryonic fission by Frost. (It is probable that some of the twins reported below under simple and euploid polyembryony also are results of monozygotic cleavage.)

Simple Polyembryony

In the gymnosperms simple polyembryony is due to the characteristic formation of a plurality of eggs from a single megaspore, and the union of these eggs with sperms. A plurality of sperms is produced from the one to several microspores which germinate within the micropyle, just opposite the point where the eggs are

² References to unpublished work of Cameron and Silow are based on communications from Dr. D. R. Cameron, Department of Genetics, College of Agriculture, University of California, Berkeley, Calif., dated Oct. 6, 1939; and from Dr. R. Silow, Genetics Department, Empire Cotton Growers Corporation, Trinidad, B. W. I., dated Oct. 20 and Dec. 19, 1939. The writer is grateful to these investigators and other research workers who have supplied unreported data or copies of articles in press.

borne. Following amphimixis, one to several independent embryos develop within the embryo sac. In the conifers, simple polyembryony has recently been reported in *Cupressus* (27), *Taxus* (87), *Sequoia* (16, 17), *Austrotaxus* (85), *Widdringtonia* (86) and *Cryptomeria* (14). It has also recently been described by Maheshwari (70) in *Ephedra* of the Gnetales.

Since neither plural eggs nor the liberation of multiple sperms in the embryo sac is characteristic of angiosperms, it is doubtful whether simple polyembryony occurs within this group. In *Santalum* (Santalaceae), *Sinningia* (Gesneriaceae) and *Mimosa* (Leguminosae) multiple embryos presumably derived from more than one egg within a single embryo sac have been reported (cf. 23). These cases are questionable, however, since their origin has been judged mainly from the fact that the developing embryos occupy the position of the egg. Although the production of more than one egg could be ascribed to cleavage, it seems more likely that in the above cases the extra embryos were derived from synergids. In *Boerhaavia* (Nyctaginaceae) Kajale (53, 54) found that in a single embryo sac accessory pollen tubes and egg-like synergids were fairly common. Dutt and Subba Rao (30) and Dutt and Krishnaswami (29) concluded that adventitious embryos in *Saccharum* (Gramineae) are derived from the fertilization of synergids by extra generative nuclei. Similar adventitious embryos have also been reported from the antipodal cells in *Allium* (Liliaceae) cited by Ernst (31) and possibly in *Alnus* (111). In all these cases the embryos are derived from a single megaspore, and supposedly by the union of haploid gametes or gamete-like cells. Their production, therefore, is comparable to simple polyembryony in the gymnosperms.

The writer is not familiar with any report on the cytological or genetical composition of the products of simple polyembryony. It is doubtful, however, that multiple seedlings in this type of polyembryony would differ greatly, since the embryos originate from a single megaspore and, presumably, in every case following amphimixis. Any differences that may exist would necessarily be of paternal origin.

Euploid Polyembryony

Under euploid polyembryony, the writer includes multiple embryos which give rise to monoploids as well as euploids. Hyper-

and hypo-ploids are also included, since in euploid polyembryony slight variations from exact multiples of a haploid probably have little significance in multiple embryo formation.

In 1933 twin plants, one with haploid and one with diploid chromosome number, were independently reported in *Oryza* by Ramiah, Parthasarathi and Ramanerjam (82) and in *Linum* by Kappert (55). Since this discovery euploid twinning has been reported as:

- 1) Haploid-haploid:³ Malvaceae—*Gossypium* (7).
- 2) Haploid-diploid: Gramineae—*Dactylis* (77), *Triticum* (57, 59, 61, 62, 76, 83, 113, 114), *Poa* (90), *Phleum* (73); Solanaceae—*Solanum* (67), *Nicotiana* (Cameron); Malvaceae—*Gossypium* (8, 40, 90, 109).
- 3) Haploid-triploid: Gramineae—*Phleum* (77).
- 4) Diploid-triploid: Gramineae—*Poa* (73, 77, 90), *Triticum* (73, 76, 77, 113, 114), *Secale* (61, 73), *Avena* (73), *Phleum* (73, 90), *Lolium* (73), *Dactylis* (90); Rosaceae—*Pyrus* (8); Solanaceae—*Nicotiana* (Cameron).
- 5) Diploid-tetraploid: Gramineae—*Triticum* (76, 113); Cruciferae—*Brassica* (42).
- 6) Triploid-triploid: Gramineae—*Dactylis* (90), *Lolium* (75), *Poa* (73).

Unclassified twins are:

Poa pratensis ($2n = \pm 52$): twins with ± 65 and ± 80 chromosomes (73).

Poa alpina ($2n = \pm 50?$): twins with ± 75 and ± 110 chromosomes (1).

— *Prunus*: twins were a haploid and a diploid-tetraploid chimera (81).

Secale cereale (a variety of hybrid origin): twins were a normal diploid and a structural hybrid (61).

Nicotiana, F_1 *N. sylvestris* ($4n$) \times *N. tomentosum* ($2n$): twins were a $4n$ *N. sylvestris* & a hybrid (Cameron).

In all the above genera, except *Gossypium*, diploid-diploid twins are the most common. Müntzing (75) found that of 2201 twin plants from 11 genera, 2106 were diploids while 77 were triploids, 11 were haploids, 2 were tetraploids and 5 had other deviations in chromosome numbers.⁴ Twins in *Gossypium* are mainly haploid-

³ This classification is compiled on the haploid chromosome number of the species concerned in twinning, rather than on the possible basic number of an euploid series.

⁴ Other deviations are mainly due to aneuploid conditions.

diploid in the 26-chromosome species, and diploid-diploid in the 13-chromosome species. In the former case Doctor Mason⁵ recently reported 28 pairs of twins all of which were haploid-diploid, while in the latter category Silow reported 10 pairs, all of which were diploid-diploid, except possibly one pair, which was probably haploid-diploid. The writer has recently found a single case of haploid-diploid twins in *Gossypium sturtii* ($n=13$). In *G. barbadense* ($n=26$) Beasley (7) reports the only case of haploid-haploid twins.

Triplets have been reported in several cases and were found in *Triticum* (114) to be triploid-triploid-diploid and in *Poa* (73) to be diploid-diploid-triploid.

It has been pointed out that identical twins are derived by monozygotic cleavage and that cytologically similar twins originate from multiple fertilization of the egg, synergids and antipodal cells. Undoubtedly many of the diploid-diploid twins and conjoined diploid twins (90, 109) occurring along with euploid twins have similar origin. Recent evidence indicates that euploid twins are also derived from a single embryo sac.

The fact that triploids occur more frequently than haploids in conjunction with diploids led Yamamoto (115) and Kostoff (61) to suggest that triploids develop from the endosperm.⁶ On the other hand, in *Trillium* (Liliaceae) Jeffrey and Haertl (46) also found that embryos are derived from the endosperm nucleus, but in this case neither the egg nor the endosperm nucleus is fertilized. Hence, haploid embryos are derived parthenogenetically and diploid embryos apogamously.

Undoubtedly many euploid twins are similarly derived from one embryo sac through various forms of reduced and unreduced parthenogenesis and apogamy. It is also probable that, following the formation of haploid and diploid nuclei of the embryo sac, fertilization by a reduced or unreduced sperm, or possibly two sperms, may occur. Thus in F_1 *Triticum vulgare* ($n=21$) \times *T. armeniacum* ($n=14$), Kasparyan (57) found twin plants with $2n=35$ and $2n=49$. The latter plant probably resulted from the union of a normal egg and either a diploid or two normal sperms. Fertiliza-

⁵ Communication from Dr. R. Silow. See footnote 2.

⁶ In the angiosperms during megasporogenesis two haploid polar nuclei are formed, which fuse to produce the endosperm nucleus. Upon union of the latter nucleus with a male nucleus a normal triploid endosperm develops.

tion by two sperms is the view favored by Kasparyan, as *T. armeniacum* does not normally form diploid gametes.

The occurrence of twin seedlings in *Oryza*, one of which was homozygotic green and the other an albino, and of twin plants with 31 and 32 chromosomes in *Medicago* led Ramiah and his colleagues (83) and Skovsted (90) to conclude that the twins were derived from two embryo sacs. Plural embryo sacs have been attributed to: a) development of extra megasporocytes in *Poa* (3), *Solanum* (58), *Medicago* (84), *Hiptage* (96), *Tamarix* (89), *Rosa* (43) and *Saxifraga* (19); b) development of sister megaspores⁷ in *Poa* (2); and to c) apospory in *Malus* (26), *Poa* (1) and other Gramineae (73).⁸

Müntzing (73) concluded from a comprehensive study of euploid twins that occasionally two megasporocytes are formed. Generally these megasporocytes give rise to haploid embryo sacs, but rarely an unreduced embryo sac is formed from the accessory megasporocyte. The majority of diploid and euploid twins are derived following fertilization or parthenogenetic development of the eggs in these embryo sacs. Such an explanation also accounts for the cytological and morphological constancy of aneuploid species (*Poa pratensis*) and for the hyper- or hypo-triploids which occur during twinning in these species. Müntzing further suggests that triploid-triploid twins are derived from unreduced sister megaspores and tetraploid twins by somatic chromosome doubling after amphimixis or possibly by doubling at the beginning of aposporic development. The latter idea is supported by the tetraploid condition of the diploid-tetraploid chimera twin seedling in *Prunus* (81), which must be due to somatic doubling after fertilization.

On the other hand, Armstrong (3) found that *Poa pratensis* was not apomictic, and with little evidence assumed that all embryo development involved haplois and amphimixis. He further assumed that aneuploid sexual types were maintained by elimination of gametes and zygotes with deviating chromosome numbers, and that

⁷ In *Ephedra* (Gnetales), Maheshwari (70) reports the occasional production of two gametophytes within the same ovule by simultaneous development of sister megaspores.

⁸ In the angiosperms apospory is generally defined as: development of an unreduced female gametophyte from cells of the nucellus or integument. Since megasporocytes are also developed from cells of the nucellus, it is possible that the first division in this classification "extra megasporocytes" should be included under apospory. However, there is some evidence that cases listed under extra megasporocytes, result in reduced gametophytes.

polyembryony increases the chances of such elimination. Åkerberg (1) strongly supported Müntzing, and emphasized that Armstrong's findings lack proof, and do not account for certain hybrid types, types with variable progenies, and euploid twins.

Unclassified Cases of Polyembryony

Cases of polyembryony were recently reported without reference to their origin in: Gramineae—*Eleusine* (6), *Festuca* (90), *Avena* (90), *Alopecurus* (90), *Lolium* (90); Musaceae—*Musa* (51); Santalaceae—*Santalum* (93); Leguminosae—*Arachis* (79), *Medicago* (90), *Lotus* (90); Solanaceae—*Solanum* (6); Cruciferae—*Brassica* (90); Compositae—*Zinnia* (90).

Recent cases of unclassified diploid-diploid twins were reported in: Cruciferae—*Brassica* (90); Linaceae—*Linum* (90); Leguminosae—*Trifolium* (90); Umbelliferae—*Daucus* (90); Gramineae—*Agrostis* (90), *Lolium* (90).

It is evident from the preceding sections that polyembryony is not always a simple process, but often complex, and that several different factors may be working at the same time, as well as at different times. Thus in *Alnus* (111) two embryo sacs may develop in the same ovule and in each of these sacs several embryos may mature; and in *Allium* (cited in 23) of five embryos in a single embryo sac one was normal, one from a synergid, two from antipodal cells and one from the integument.

True and False Polyembryony

The term polyembryony is definable as the production of two or more embryos within an ovule. Ernst (31) distinguishes two classes of polyembryony: true and false. The production of plural embryos within, or by projection into, a single embryo sac is designated as true polyembryony. False polyembryony is the production of plural embryos derived from several embryo sacs. In the latter case multiple embryo sacs are derived from: *a*) megasporocytes in different nucelli; *b*) two or more megasporocytes, sister megaspores, *etc.*, in the same nucellus; and *c*) from the normal megasporocyte and apospory in the same nucellus.

It appears that the distinction between true and false polyembryony is purely an arbitrary separation and that a more natural or physiological distinction would be more appropriate. In many

cases true polyembryony involves apomixis, which in certain plants appears very largely dependent on some growth stimulus due to pollination and possibly fertilization. It is very likely in cases where two embryo sacs are contiguous that any outside stimulant would have equal or nearly equal effect in both embryo sacs. Likewise it is probable that stimulants produced in one embryo sac affect the other. Embryo sacs derived from the same nucellus are generally adjacent and in the same or nearly the same stage of development. Chapman (19) recently found that such embryo sacs lay side by side unseparated, or separated only by two or three layers of crowded and pushed together nucellus cells. In the formation of euploid embryos from plural embryo sacs several factors are involved which undoubtedly work together, as well as independently. These conditions, as well as the evidence that the nucellus influences the developing embryos, leads the writer to believe that multiple embryos formed within the same nucellus are cases of true polyembryony.

If the preceding modification of Ernst's classification is accepted, then false polyembryony would be limited to those cases in which multiple embryos are derived from different nucelli. Such cases of polyembryony are readily separated into: *a*) those in which more than one ovule develops in a normally 1-ovulate seed-like structure or locule, and *b*) those which originate from fusion of ovules or developing ovules. Cases of the first class of false polyembryony commonly occur in such drupes as almonds, plums and peaches. Although these drupes normally have a single seed within the hard seed-like endocarp, sometimes two or even three are found. During formation, each of the plural seeds develops from a separate ovule, provided with a separate funiculus and micropyle. Similar cases of such false polyembryony have recently been reported in *Coffea* (63) and *Sandoricum* (47, 49).

The second class of false polyembryony resembles the first in that plural embryos are undoubtedly derived from separate ovule initials of the carpel. In this case, however, occasionally two of the developing ovules unite to form what appears to be a single composite ovule. The proximity of the developing embryos is apparently entirely dependent upon the degree of formation at the time of fusion. Fusion may take place early in development so that there is only a single micropyle, or later in development when there are two micro-

pyles. In the majority of cases, the forming embryos are well separated by nucellar tissue, tegmen, and other tissues of the ovule. The final seed may appear single or double. Such fused ovule development has recently been clearly depicted by Howard (42) in *Brassica* and reported in *Oryza* by Kondô and Isshiki (60).

It is evident that the preceding two classes of polyembryony are truly false, since the embryos are derived from different ovules. In such cases the effects of stimulants are undoubtedly considerably more limited than in cases where plural embryo sacs are derived from a single nucellus. In *Gossypium*⁹ plural seedlings derived from fused ovules are fairly common. No euploid plants have been found among such seedlings.

FREQUENCY OF POLYEMBRYONY AND DEVELOPMENTAL COMPETITION OF PLURAL EMBRYOS

It is well known that polyembryony is more common in some species, varieties and strains than in others. Thus in *Gossypium barbadense* the frequency of twins in one strain of Sea Island cotton is approximately 1 in 300 seeds,¹⁰ whereas in another strain it is 1 in 500 seeds (7). In other 26-chromosome cottons plural seedlings are rather rare (40, 90, 109), while in the 13-chromosome species twins are very rarely found. In the latter category Silow recently reported 1 pair of twins from 40,000 seeds of Asiatic cottons. Similar results have been reported in *Citrus* (78, 99), *Poa* (1, 3, 73) and other genera (73).

Polyembryony in the gymnosperms is so prevalent that only an exhaustive study of the literature would make it safe to venture an estimate of its extent. Hence, the writer merely points out that in certain conifers it has been estimated that in a single ovule between 1 and 200 potential embryos are possible on the basis of simple polyembryony (11, 86) and between 1 and upwards of 27 per zygote by cleavage polyembryony (11, 12).

In the angiosperms, sporophytic polyembryony appears to be considerably more limited in its taxonomic distribution than other types of polyembryony. In the majority of plants where it occurs, however, the frequency of plural embryos is extremely high. In certain varieties of *Citrus* (34, 78, 99, 105) and *Mangifera* (52) nearly every seed is polyembryonate and approximately 30 embryos

⁹ R. Silow, and the writer, not previously reported.

¹⁰ Dr. Mason, see footnote No. 5.

have been observed in a single seed. Frost (32) found that in 10 varieties of *Citrus* the mean number of embryos per seed varied from 1 to 6.5. The numbers of sporophytic embryos per seed recently reported in other genera are: *Eugenia* (44) 1 to 20, *Hiptage* (96) 1 to 7, and *Myricaria* (104) 1 to 5. In *Murraya* (18) a single sporophytic embryo, along with the gametic embryo, occurs about once in 120 seeds.

Among more than 1000 *Citrus* hybrids, Frost (32) found 10 or 11 cases of 2 hybrid seedlings from a single seed. Since these twin seedlings were identical, Frost concluded that they were derived by cleavage. Traub and Robinson (105) confirm the frequency of polyembryonate hybrids observed by Frost, but they report a maximum of 4 hybrids per seed. Cleavage of a single zygote undoubtedly accounts for the monosomic triplets among 6215 *Nicotiana* seedlings reported by Cameron.

Since plural embryos are of rare occurrence in most species, the actual frequency of polyembryony is usually indicated only by the relative occurrence of plural seedlings. Furthermore, since it is generally impossible to determine the probable origin of the plural seedlings the frequency includes all types of polyembryony. The percentages of plural seedlings have recently been reported as approximately 0.01 in *Oryza* (83), 0.02 in *Avena* (73), 0.05 in *Musa* (51) and *Nicotiana*, 0.02, 0.09, 0.20 and 0.22 in *Triticum* (61, 73, 114, 115), 0.03, 0.08 and 0.14 in *Secale* (61, 73), and 8.00, 11.00, 35.00 and 42.0 in *Poa* (3). In the sexual types of *Poa*, Åkerberg (1) found that 3.4% of the seeds produced twins, while the seeds of apomictic types gave rise to 9.8% of twins.

As indicated in the preceding sections, in the majority of genera about 5% of twin plants are euploid. However, in some types of *Poa* from 9 to 12.7% (1, 74) of the twin plants are euploids, while in certain strains of *Triticum* (61) and *Gossypium* the frequency of haploids among twins is approximately 25% and 50%.

In all instances where relatively large numbers of embryos are formed, there is a struggle for existence. Consequently the embryos vary in size and completeness of development, and usually only a few germinate. Buchholz (9) points out that *Ginkgo* is the only gymnosperm which occasionally matures more than one of its several embryos. However, more recent work indicates that plural seedlings in this group of plants are as frequent as in the angio-

sperms. Thus, Skovsted (90) reported twin seedlings in *Pseudotsuga*, and Clare and Johnstone (20) found that approximately 1.59% of the seeds of three species of *Pinus* produced twin plants. In the angiosperms, Frost (34) and Torres (99) found that the mortality among *Citrus* embryos increases in direct proportion to the number of embryos in the seed. Highly polyembryonate seeds of *Eugenia* (45), *Myricaria* (104), *Citrus* (34) and *Mangifera* (48, 52, 101) respectively produce only 1 to 2, 1 to 2, 3 to 4, and 2 to 8 seedlings.¹¹

Traub (103) has suggested that environmental conditions may materially influence the number of seedlings which develop and survive. In certain *Citrus* species he found that by decreasing the food supply the number of seeds producing plural embryos was from 51% to 100% below the expected number. Juliano (50) lists several varieties of *Mangifera* which are monoembryonate in certain localities but polyembryonate in others. Whether this difference is due to environmental differences or to cross-pollination is unknown. In the gymnosperms, Clare and Johnstone (20) found evidence that the number of embryos developing depended upon the degree of endosperm elimination.

According to Müntzing's (73) theory of the production of euploids from reduced and unreduced embryo sacs, triploids are weaker because: *a*) the normal reduced embryo sac probably has a more favorable position in the nucellus and will be better nourished, and *b*) the relations between chromosome numbers of embryo, endosperm and surrounding tissue in the unreduced embryo sac equals $3n$, $5n$ and $2n$, respectively, instead of the normal $2n$, $3n$ and $2n$. In early works Müntzing showed that such deviations from the normal ratio have a pronounced effect on the vitality of the embryos. That the position of the developing embryo is an important factor in its chances of survival is suggested in *Citrus* by Frost (32) and Traub and Robinson (105).

Buchholz (15) recognizes two types of cleavage polyembryony in the gymnosperms, determinate and indeterminate. In determinate cleavage one embryo, usually the terminal one, is more

¹¹ Prof. H. J. Webber, University of California, Citrus Experiment Station, Riverside, California, states in unpublished notes on the mango in Florida, 1932: "In 25 seeds . . . it was found that the number of embryos per seed ranged from 2 to 8 with an average of 4.24 embryos per seed. In the seed with the highest number of embryos, 8, all had germinated. . . ."

favorably situated than the others and generally survives. In indeterminate cleavage there is no indication that a particular embryonic unit has a distinct advantage, and any one of several embryos may become the successful embryo. On the other hand, the favorably located gametic embryos of highly polyembryonate *Citrus* clones are frequently eliminated. In this case, however, since Frost (34) has shown that the gametic seedlings are inherently weaker than metromorphic seedlings, it is apparent that sexual embryos are less likely to stand competition. Traub (103) listed other factors that may operate in eliminating plural embryos, such as nature of food supply, moisture supply of developing embryos, temperature, age of seed, seed maturity and desiccation of seed.

/ CAUSES OF POLYEMBRYONY ✓

With reference to polyembryony in the gymnosperms Buchholz (10) stated that "cleavage polyembryony is a feature of the embryogeny which must have had its origin before the developmental selection within an ovule had become adjusted, under conditions when only one egg at a time was fertilized." Previously (9) referring to cleavage in the conifers he wrote: "This character tends to be modified or eliminated, reverting to simple polyembryony as we advance along several phylogenetic lines and is lost by the time the level of the angiosperms is reached."

Since the majority of cases of plural embryo formation in the angiosperms usually involve apomixis, the causes of polyembryony and apomixis are generally considered together. In the early literature apomixis and polyembryony were regarded as very unusual and were considered as: *a*) a gradual replacement of sexual reproduction, caused by weakening of sexuality; *b*) early stages of the development of something new; *c*) a primitive feature; *d*) a development by the plant to increase its capacity for dispersal; *e*) merely somatic buds which due to stimulation develop into embryos.

Recently polyembryonate plants have been frequently associated with meiotic irregularities (3, 66), polyploidy (8, 22, 40) and hybridization (57, 67, 83). Ernst (31) has pointed out that hybridization is the primary cause of chromosome aberrations and of replacement of sexual reproduction by apomixis. Polyembryony in a triploid apple was recently attributed to similar causes by Dermen (26). On the other hand, in *Eugenia*, Pijl (80) found that the

chromosome condition did not point to polyploidy or hybridization as a cause of polyembryony. Pijl refers to nucellar embryony of *E. jambos* as a reduced form of normal reproduction, and to apogamy, apospory and other similar occurrences as intermediate forms.

In the preceding section it was shown that polyembryony is more prevalent in certain strains than in others, and that physiological changes effect the frequency of plural embryos. Such evidence clearly supports Kappert's (55) suggestion that although polyembryony is constitutional, environment determines the degree of expression. The fact that highly polyembryonate strains frequently segregate from hybrids derived from less polyembryonate parents (36, 107, 108), indicates that polyembryony is of zygotic origin. In *Linum* Kappert (55) concluded that polyembryony is a recessive character probably conditioned by a series of multiple factors which are merely brought together in suitable recombinations following hybridization. Similar views were indicated by Sokol'skoja (91) and Frost (36) with regard to *Citrus* by Ramiah and colleagues (83) regarding *Oryza*, and by Yamamoto (116) in his studies of twins.

POLYEMBRYONY AND PHYLOGENY

Haploid, triploid and tetraploid twin plants are rapidly being utilized in solving many agricultural and botanical problems. Since their discovery in 1933, they have been subjected to extensive histological (67, 77), cytological (59, 62, 67) and morphological (40, 73, 90) studies. From these studies and similar work with diploid plants and hybrids, significant phylogenetic conclusions have been drawn (1, 7, 40, 73, 109).

Darlington (25) stated, "In about half the species of the angiosperms the gametic chromosome number is a multiple of that found in some related species, the chromosomes being themselves comparable in the two forms. From this alone it is clear that they owe their origin to polyploidy. . . ." It is known that most polyploid species and complexes (94, 95) are more widely spread in their distribution than diploid species or diploid complexes. Stebbins (94) concluded, "The evidence from the plant kingdom as a whole, . . . suggests that polyploidy has been most important in developing large, complex and wide-spread genera, but that in respect to the major lines of evolution, it has been more important

in preserving relics of old genera and families than in producing new ones." Since there are no natural methods known which produce polyploids more frequently than twinning, undoubtedly polyembryony has performed an important role in the origin of polyploid species. Furthermore, since polyembryony frequently involves hybridization, it is evident that this origin has been significant in the wide distribution of these polyploid species.

The variability of gametic seedlings in polyembryonate forms of *Citrus* led Frost (34) to believe that these forms are extremely heterozygous. He concluded that "Nucellar embryony, by providing a natural means of asexual multiplication, has doubtless been very favorable to the perpetuation of heterozygosis, whether this has arisen through gene mutation or through hybridization or in both ways." Toxopeus (101) concluded that polyembryony in *Citrus* has favored the preservation of new types, but has hindered their origin. The fact that nucellar embryony constitutes a hereditary character, has frequently led to the belief that it may be utilized in systematic classification.¹² Thus in *Citrus* the fact that pummelo (Shaddock) and grapefruit, generally classified as one species, are monoembryonate and polyembryonate, respectively, led Torres (99) to conclude that each is of specific rank. Sokol'skaja (91) held similar views regarding *Citrus*. However, it should be borne in mind that the degree of expression of polyembryony has been proven to depend on environmental conditions.

In the gymnosperms polyembryony is an embryological character that has frequently been employed in determining possible relationships. These studies (13, 21, 27, 28, 72, 87, etc.) mainly involve the occurrence and frequency of simple and cleavage polyembryony, and the stage, place and method of the latter. It has been pointed out that Buchholz recognized two types of cleavage polyembryony: indeterminate and determinate. He regarded cleavage as a primitive character which during evolution gradually became reduced and replaced by simple polyembryony. Buchholz (15) listed the

¹² Note received from Dr. W. T. Swingle, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C., April 7, 1940 states "Antoine de Jussieu discovered polyembryony in a plant now called *Esenbeckia pilocarpoides* H.B.K. and made a new genus which he called *Polembryum*, naming the species *P. castanaecarpum* A. Jus. in Mém. Mus. Paris, 12: 519, pl. 28, 1825. Nine years later Schott named another species of *Polembryum* in honor of Jussieu, calling it *P. Jussieu*; it is now called *Esenbeckia grandiflora* Mart. Schott published his species in his paper Rutaceae, Fragmenta Botanica, Vienna, plate 6, 1834."

steps in this evolution as: *a*) Indeterminate cleavage (*Pinus*, *Cedrus*, *Tsuga*, *Biota*, *Cryptomeria*, etc; *b*) determinate cleavage (*Pseudolarix*, *Dacrydium*); *c*) simple polyembryony exhibiting definite traces of determinate cleavage (*Cryptomeria*, *Chamaecyparis*); and *d*) simple polyembryony without traces of determinate cleavage. Although limitations of space prohibit further discussion of the many relationships indicated by polyembryony in the gymnosperms, the writer feels justified in calling attention to the fact that as early as 1920 Buchholz (9) pointed out that the occurrence of cleavage polyembryony in the Gnetales connected this group with the Coniferales, rather than with the Cycadales. This connection has recently been confirmed by Cook (21) and Maheshwari (20).

POLYEMBRYONY AND AGRICULTURE

Advantages

Haploids and polyploids are fully as valuable to agricultural as to phylogenetic studies. The possibilities of obtaining homozygous diploids from haploids, and the importance of such pure lines in genetical and plant breeding work has often been discussed (22). Haploids are produced more frequently by twinning than by any other known natural or artificial means. Recently developed methods of doubling chromosome numbers (61) have greatly increased the chances of obtaining diploids from haploids. Such use of haploid twin plants has recently been discussed by Harland (40), Webber (109) and Beasley (7) with reference to cotton and by Kostoff (61) with reference to rye and wheat. Many of our most important agricultural plants, as cotton, tobacco, wheat and potato, are polyploids, some of which undoubtedly originated through polyembryony. Müntzing (74) found that polyploid twin timothy plants and their progeny had a higher weight than normal plants. He also obtained from twins (73) several polyploid pasture grasses that were "gigas" in character. Since these variants may give rise to high-yielding varieties, Müntzing considered twinning a valuable aid in plant improvement.

To maintain fruit size and yield in *Citrus* Webber (107) found it was extremely important that rootstock and scion be physiologically congenial. Swingle (97) and Webber (106, 108) pointed out that in *Citrus* the main value of polyembryony is provision of a means by which large numbers of genetically uniform stock seed-

lings may be easily and cheaply obtained. Although Webber found that a considerable proportion of the gametic seedlings could be eliminated in stock nurseries by discarding approximately 10% of the smallest seedlings, Torres (99) believed that it is impossible to pick out and eliminate such variant seedlings. Frost (34) showed that gametic seedlings are usually weaker than nucellar seedlings. Gandhi (37) stated that in India it is highly important that rootstocks of *Citrus* and mangoes be standardized, and suggested that polyembryony be utilized to this end. Oppenheimer (78), Toxopeus (101), Lapin (68) and others supported Webber's conclusions, while Torres (100) further found that in the Philippines it was of practical value to provide certain *Citrus* hybrid seedlings with double root systems by inarching nucellar seedlings.

In 1932 Swingle (98) pointed out the reduction of spines in old *Citrus* clones and postulated that clonal varieties which have become senescent are rejuvenated during nucellar embryony. His hypothesis of "neophytosis" assumed that embryo sac stimulants effect this rejuvenation. Traub and Robinson (105) found no proof that *Citrus* clones are subject to senescence and concluded that "supposed rejuvenation is explainable as a permanent genetic factor change due to a nutritional effect or may be better explained by Frost's theory of 'islands' of mutating tissue in the nucellus." Frost (35), however, recently reported that in young *Citrus* clones such juvenile characters as vegetative vigor and extreme thorniness gradually decline with increasing age of the tree and with repeated clonal propagation by budding. Frost observed that as these juvenile characters became less prevalent, the trees became earlier bearers, the tendency to flower and set fruit increased, and the fruits contained more seeds. Since the juvenile characters were immediately reestablished during nucellar embryony, Frost concluded that the change was akin to senescence, and rejuvenation was not due to genetic changes. He also showed that clonal senescence was not due to diseases or similar accidental causes. Hodgson and Cameron (41) found similar nucellar rejuvenation in *Citrus* and suggested that it might be utilized to invigorate polyembryonate clones and reduce their seed content. They also pointed out that premature decline of Eureka lemon trees, caused by lack of scion vigor, may possibly be eliminated by asexual seed propagation of the variety.

Disadvantages

Webber (107) also pointed out that the main disadvantage to agriculturalists of polyembryony in *Citrus* is inability to distinguish nucellar and hybrid seedlings in early stages of development. In hybridization studies this necessitates the expense of growing large numbers of plants to secure a few hybrids. To overcome this handicap the necessity of utilizing monoembryonate or only slightly polyembryonate plants as the maternal parent has been stressed by Lapin (69), Oppenheimer (78), Toxopeus (101) and Traub and Robinson (105) with reference to *Citrus*, and by Juliano (48) with reference to mango. "It has been mentioned that nucellar embryony hinders the origin of new types. It also greatly handicaps genetical analysis. Frost (34) found that in polyembryonate forms of *Citrus* the variability of gametic seedlings was so great that positive identification of the effects of single gene differences was extremely difficult or impossible. He concluded that somatic (bud) variations and differences among nucellar seedlings from the same parent suggest gene mutation, and that the small number of nucellar seedlings which are strikingly different from their parent suggests the presence of a highly "variable" or unstable gene. Frost also suggested that the frequent occurrence of somatic variations in polyembryonate *Citrus* varieties is "due fundamentally to gene shifting (crossing-over, reduplication, etc.), and possibly gene mutation in extremely heterozygous chromosome complements, which readily permit such changes to produce perceptible somatic effects."

It should also be mentioned that tetraploid *Citrus* plants, like many other polyploid forms, are inferior in quality and yield. Frost (36), however, concluded that these tetraploid plants crossed to ordinary diploid plants, might produce triploid or modified triploids that would probably be practically seedless.

Webber¹⁸ has called the writer's attention to the fact that while rejuvenation may have the possible values mentioned in the preceding section, the accompanying thorniness is a disadvantage. He also stated that he and other early *Citrus* breeders, lacking knowledge of rejuvenation and senescence, introduced several new varieties which later became indistinguishable from the original clone.

In Iowa and adjoining States, Martin and Watt (71) found that from 50% to 80% of the ovules of red clover exhibited polyembry-

¹⁸ Prof. H. J. Webber (unpublished).

ony or irregularities leading to polyembryony, which caused shedding of the flowers or young pods. They suggested that the reduction of seed capacity of red clover in the vicinity is probably due to these abortions.

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COLCHICINE POLYPLOIDY AND TECHNIQUE

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INTRODUCTION

The use of the chemical colchicine as a means of chromosome doubling has opened a large reservoir of possibilities in plant breeding work. The fact that any numerical change in chromosome number fundamentally entails a mutation which may be expressed in a number of characters of the experimental plant indicates the significance of the above statement. Blakeslee (7), referring to polyploidy induced in taxonomic hybrids as a method of evolution of species in nature, states that with the colchicine method "We now have an opportunity to make new species to order," that "The possibilities in the way of new forms of economic value seem very great," and "We now no longer have to wait ages for the chance hybridization between species and the later rare spontaneous doubling of their chromosomes in order to secure such superior varieties [referring to wheat, oats, timothy, tobacco and cotton]. We can now make them up to order." Vavilov (88) states: "The possibilities opened up by the artificial induction of amphidiploidy, *i.e.*, of chromosome doubling in hybrids, are immense. Genetics is entering a new era of extensive application of distant hybridization, at least in the case of plants." Many successful results in inducing polyploidy by colchicine treatments, in the brief time since the inception of this technique in 1937, support the above statements.

Without going into details, herein is described briefly what is meant by polyploidy and some other related phenomena. In both animals and plants a numerical change of chromosomes involving an addition of at least one set of chromosomes, a basic set or a genom, $1x$, is termed polyploidy; and plants with one or more extra sets of chromosomes in addition to the two sets, diploid number, $2x$, normally present in the somatic tissues of higher plants, are recognized as polyploids. In lower plants such as liverworts and mosses in which the major tissues are gametophytic and normally have but one set of chromosomes (monoploid, $1x$), the addition of one or more sets makes the plant polyploid.

According to Müntzing (61), more than half of all angiosperms found in nature are polyploids and some others have been produced

experimentally. Thus the magnitude of the importance of polyploidy becomes apparent. So frequently have these plants been shown to have desirable characteristics associated with their polyploidy that we find full justification for the profound interest that the use of colchicine in inducing polyploidy has aroused among plant breeders the whole world over. The fact that the technique of applying the drug has proved to be comparatively simple may also have contributed to this interest.

Investigations to date on colchicine-induced polyploidy have been chiefly concerned with the mechanics of doubling the chromosome number in a great many plants (7). Since the use of colchicine as a reliable means of inducing polyploidy has now been definitely established, and a number of practical techniques in the application of the chemical have been developed, more attention will undoubtedly be given to specific problems in plant breeding. These include sterility, particularly following hybridization; production of polyploids for hybridization with known polyploid plants; development of plants for wider distribution and for adaptability to a different environment; introduction of quantitative as well as qualitative changes as result of polyploidy, *etc.*

A general discussion of the subject of polyploidy, the value of polyploids in agriculture, and the significance of polyploidy in evolution are omitted from consideration in this paper. For such discussions the reader is referred to cytological books by Sharp (82) and Darlington (12), and to articles by Müntzing (61) and Anderson (4). This paper will present the history of colchicine-induced polyploidy as applied to plants, the cytological mechanism of polyploidy induced by colchicine, the technique of colchicine application and some of the results thereof. Polyploidy induced by other chemicals is not discussed here. However, references to such agents are given mostly in a separate list of literature following literature citation. Fyfe (38) has recently presented a review of the work on colchicine-induced polyploidy, comprising 38 articles, and Havas (45, 46) has discussed the uses to which colchicine has been put, aside from inducing polyploidy in plants, and has listed 52 references.

HISTORY

Successes recorded in artificially inducing polyploidy until recent years have been mainly in certain cells in contradistinction to poly-

animal tissue." Eigsti also had the opportunity of seeing some colchicine-treated animal material of his colleague, Mr. E. L. Lahr, "in which," he says, "mitotic figures were more abundant than in the prepared sections from untreated tissue. . . . This observation suggested many possibilities." Eigsti's cytological observations evidently culminated in the successful plant polyploidy work by Blakeslee and Avery (8), but his article on his cytological findings appeared after the practical results were reported by the above authors, who acknowledged Eigsti's suggestion to them of the use of colchicine in polyploidy.

The initiation of Nebel's work (64, 65) is also linked with the animal work conducted by Allen and associates. Nebel and Ruttle (67) state that Dr. D. F. Jones, of the Connecticut Agricultural Experiment Station at New Haven, who was informed of the colchicine work at Yale, mentioned to them the possible value of "the use of colchicine as an agent which might lead to induced polyploidy." Thus Nebel also apparently initiated independently the colchicine technique directed toward the production of polyploid plants.

The history thus presented makes it evident that in recent years the use of colchicine applied to biological problems was initiated in Prof. A. P. Dustin's laboratory in Brussels, Belgium, in 1934, and that indirectly the cytological tests performed with the drug by Eigsti and by Nebel were suggested by the work of Allen and associates. These investigations culminated in successful induction of polyploidy in plants by Blakeslee and Avery, by Nebel and Ruttle, and by many others following. This, in the main essentials, is also the history presented by Wellensick (92) as to the origin of the use of colchicine as a polyploidizing agent for plant material.

COMPARATIVE CYTOLOGICAL EFFECTS OF TEMPERATURE AND OF COLCHICINE TREATMENTS

Following the preliminary report of Blakeslee (6) on the practical results in polyploidy from the use of colchicine, the cytological aspects of the problem were studied by many investigators and it became evident that there was a general agreement among cytologists as to the mechanism involved.

Investigations have shown that in animal cells colchicine usually arrests chromosome development at metaphase, and the cells, after

reaching this stage of chromosome development, may die (1, 2, 3, 10, 13, 26, 27, 29, 30, 31, 55, 56, 57, 70). In plants, application of colchicine in proper dosage, instead of destroying the cells, results in doubling of chromosomes, *i.e.*, in polyploidy. A considerable number of independent cytological investigations by Nebel (64, 65), Eigsti (34) and many others in this as well as in other countries (21, 39, 40, 41, 47, 52, 53, 59, 68, 83, 89, 90), have shown the principal cytological factors involved in the phenomenon of plant polyploidy. A review of the cytological effects of temperature treatments (21, 77, 78) on cells will facilitate an understanding of colchicine effects.

The specific technique devised by Randolph in the use of high temperature was probably the principal contributing factor in his success in inducing artificial polyploidy. It had been known earlier that chromosomes could be doubled in plant material subjected to such physical treatment, but practical results were not obtained until he applied the technique to the single-cell stage, mainly the fertilized egg of maize, just at the time when the zygote was to divide and begin growing into an embryo. By this technique he was able to get a small percentage of seeds which developed into whole tetraploid plants.

The cytological implications of this technique were studied by Sax (77, 78) and Dermen (21). These studies were made on the meiotic phase of *Rhoeo* and *Tradescantia*, and only incidentally on the mitotic phase of *Rhoeo* (21). It appeared that by the temperature changes, either high or low, the chromosomes of the microcytes at metaphase were clumped together. At either first or second meiotic metaphases the chromosomes failed to move to opposite poles; instead, they remained aggregated and formed a single nucleus by going into the interphase (resting stage) soon after the plants were subjected to treatment. When plants were moved to normal temperature the affected cells resumed their development; but since divisions were prevented by the treatment, cells would contain the double number of chromosomes. By a temperature change only one meiotic division is prevented, so that when the affected microcytes resume their development they produce two diploid microspores instead of the four monoploid microspores normally produced. Doubling of chromosomes usually occurs but once. Only by a close succession of temperature treatments may both the

meiotic divisions of microcytes be prevented, thus producing tetraploid microspores (21).

As we will see below, this restricted polyploidy effect following a temperature treatment is a major difference from that effected by colchicine technique. Temperature treatment also affects the chromosomes of microcytes at leptotene phase, which also, like the affected metaphase microcytes, develop into diads containing the doubled number of chromosomes; while the microcytes from pachytene to diakinesis stage come through without apparent effect and divide meiotically as usual (21).

When the examination of anthers of the treated material containing microcytes at first metaphase was continued, there were found anthers in which some of the microcytes were tetraploid instead of diploid (21). These anthers were at a premeiotic stage when temperature treatment was given. The cells in the young anthers are like somatic cells in the other vegetative parts of the plant, and like any other vegetative tissue these anthers contained a very small percentage of cells at the mitotic metaphase at one time. It seemed, therefore, that by temperature changes, only those somatic cells are affected which are at divisional stages when treated, whereas in the meiotic stage the effect is also on leptotene as well as on metaphase chromosomes. It should be pointed out that in the anthers the microcytes, beginning from leptotene phase, are almost uniformly at similar stages of development; therefore, when any change occurs in the anthers, all the microcytes are affected quite uniformly. The difference between anthers containing microcytes at meiotic stages and those of premeiotic stage accounts for the difference in the number of the affected cells in those anthers.

When the temperature technique was applied (in the form of water at 1° to 3° C., or warm water at 35° C.) to some newly germinated seedlings of apple, cherry, lima bean and peach, there was found only a limited number of tetraploid cells scattered in the root-tip sections examined. Cold treatments, both in air and in water (21), were as effective as warm treatments without the injurious effects that may follow warm treatments.

In general, growth processes are almost completely arrested in the treated material shortly after it is subjected to the extreme temperatures. The cells that are in divisional phase, failing to divide, enter into prophase and, along with the great mass of vegetative as well

as meiotic cells at prophase, remain at that phase. When the experimental material is transferred to normal environment and growth is resumed, the only change that can be observed is in those cells that were at divisional or presynaptic stages, the change consisting of chromosome doubling only in those cells, thus limiting the number of tetraploid cells to a small percentage (21). This extent of the polyploidy effect forms the basis of the most important difference between the results of temperature and of colchicine treatments.

In colchicine treatment, growth is not stopped to any appreciable degree. One aspect of growth is altered, as indicated above. Cell division into sister cells is prevented by specifically affecting the mechanism of division, while the chromosomes, genetically the most important constituents in the cells, continue to develop. They split into sister chromosomes, but remain together and together go through the nuclear phase. In consequence, chromosomes are doubled in number and the affected cells grow proportionately. The processes of such an increase in number of chromosomes and cellular increase in general may continue as long as the material is exposed to colchicine, and result in enlarged cells with huge chromosome numbers (21, 22, 23, 47, 52, 68, 89). This continues until some other factors intervene and limit the growth (22 or 23).

X The apparent difference between the temperature method as a polyploidizing agent and that of the colchicine method lies, therefore, in the fact that temperature effect is only temporary and is limited to certain stages of chromosome development, while colchicine effect may be considered extensive and unlimited. Colchicine in solution is diffusible into plant tissues, and entering there it can exert its effect on meristematic cells as long as it is maintained at an effective concentration. Colchicine, like temperature, does not have any effect on resting cells, and like temperature, its effect is specific to metaphase and leptotene.) However, its effect can be extended as long as it remains present in meristematic tissues. Thus when resting cells of such a tissue enter the division phase they are immediately affected by colchicine present in them. Therefore, by colchicine, theoretically a whole tissue may be transformed from diploidy into polyploidy if that tissue is subjected to treatment during a specified time, determined by the period required to complete the divisional cycle of a meristematic cell, assuming that

it will take the same amount of time for every cell of a meristematic tissue. Such a transformation is not possible by the temperature method.

REACTION OF ANIMAL CELLS TO COLCHICINE

It was shown earlier that the use of colchicine as a polyploidizing agent was derived from its use first with animal material. The most common occurrence in animal cells after an exposure to colchicine was that in the treated material cell development was arrested at metaphase; thus in such material there would be found many more "metaphase" figures than in the untreated material (10, 13, 26, 27, 29, 30, 31, 55, 56, 57). The affected cells do not develop further but become "pycnotic" (56) and degenerate (13). Nebel and Ruttle, in their first report (67) on colchicine polyploidy, were perhaps the first to report chromosome doubling in animal material, such as artificially inseminated eggs of *Arbacia punctulata*, and remarked that "Colchicine inhibits spindle formation. In dividing cells of animals and higher plants, cells with the doubled chromosome number are thus formed." Just recently Pincus and Waddington (70) have also reported induction of tetraploidy in single-celled ova of rabbits, with the use of colchicine solutions varying from 0.000041% to 0.0041%. These are, however, the only references¹ that have come to the writer's attention concerning the doubling of chromosome number in animal material from colchicine effect.

It has been experimentally shown that in the somatic cells of both plant and animal material, spindle formation was inhibited (in plants: 34, 47, 52, 67; in animals: 13, 67, 70); thus chromosome division into two nuclei and cell division into two cells was prevented. Significantly, while in animal cells further metamorphosis of chromosomes was stopped and cellular degeneration ensued, in plants the metamorphosis of chromosomes appeared to be not affected; but sister chromosomes being left together in the affected cells, chromosome number was doubled. Even though doubling of chromosome number has been induced in animal cells (67, 70), as yet the cells have not survived very long and have not developed either into a polyploid individual or into polyploid tissue.¹ This dif-

¹ The following note by Edna Higbee, bearing the title "Some results of colchicine injections," appeared in *Science* 92: 80, after the present article was submitted for publication. For the significance it might have in the near

ference of effect makes possible the utilization of colchicine-induced polyploidy in plants.

REACTION OF PLANT CELLS TO COLCHICINE

In root-tip material, immediately following colchicine treatment, metaphase chromosomes take on a characteristic appearance (52, 68). The sister chromosomes are found attached at the region of the spindle attachment, while the arms of the chromosomes at both sides of this attachment shorten and are opened up into X-like forms. It is normally at this polar constriction that the separation of sister chromosomes takes place; but, apparently, colchicine not only prevents the functioning of the divisional mechanism; it also slows down the metabolic processes, thus bringing about also a delay in separation of sister chromosomes (52).

Following the prophase stage, when the nuclear membrane disappears and chromosomes are released into the cytoplasm, normally the chromosomes first orient in a definite metaphase plate formation, then anaphase and telophase follow. In treated material these phases are not present and no normal metaphase plates are formed (83), polarity of metaphase chromosomes being characteristic of divisional phenomena in the normally dividing cells (21). Many investigators have observed an unusual increase of metaphase cells in both plants (52, 68, 83) and animals (10, 13, 26, 27, 29, 30, 31, 55, 56, 57) following colchicine treatments. This increase is interpreted by Dustin and associates to be a result of the stimulating effect of colchicine. But it has been shown by Brues (10) and Ludford (57) that the apparent increase of what resembles metaphase is due to the fact that "the cells can proceed so far with the division process, but cannot complete it" (57), and is not due to excitation or stimulation by colchicine. Levan (52) considers the delay in the division of the centromere "partly at least, the cause of

future on polyploidy which may be induced by colchicine in some animals, the note is presented here in its entirety:

"Injections (Peter Gray method) of 0.02 cc of a 0.0001 per cent. solution of colchicine used on developing 24-hour chick embryos have shown the following results: (1) four of 20 injected eggs hatched, two males and two females; the hatched chicks have now reached the age of 9½ months, except for one hen which has been sacrificed for histological studies. (2) The combs and wattles in both sexes are abnormally large, approximately of twice the size of normal chickens. (3) Two of the tail feathers of the roosters have become greatly elongated. (4) The hen kept in the cage with one of the roosters lays non-hatching eggs at the rate of one every two or three days."

the apparent impression of mitotic stimulation, which is always found after c-treatment [colchicine treatment]." . . . "The pro-phases arrive at metaphase and are kept at that stage for a long period until the centromere finally divides." However, it is probable that the delay in centromere separation is of indirect rather than direct colchicine effect. ✓ On the other hand, the elimination of typical metaphase formation and the elimination of anaphases and telophases may account for an *apparent* increase of metaphase figures (mostly distorted) (83), but *actually* these metaphases represent the sum total of anaphase and telophase figures which would have been formed normally but are prevented from formation. Thus the chromosomes, following prophase, instead of going through various stages of metamorphosis, as separation (anaphase) and condensation (telophase), before their entrance into the nuclear stage after late telophase, go through these transformations during what appears to be a colchicine-metaphase, hence the *apparent* and *not actual* "increase" of such a phase following a colchicine treatment.

A hypertrophy generally results in growing regions, usually in root-tips, especially when newly germinated seedlings are exposed to colchicine effect for a considerable time so that the chromosome number of some nuclei increases more than once. Therefore, it has been assumed in general that such hypertrophy is directly associated with an increase of nuclear as well as cellular volume (21, 22 or 23, 52). However, O'Mara (68) states: "The hypertrophy usually associated with treatment was found not to involve the meristem but the region of elongation." Similarly, hypertrophy has been observed in the coleoptile of treated young grass seedlings where supposedly the growth is primarily by enlargement rather than by cell division. Such an effect described by O'Mara may indicate that the effect of colchicine is not confined to the disturbance of the divisional mechanism of cells but extends to some other component parts of the cell, which may result from the somewhat toxic effect of colchicine observed, especially when some higher concentrations are used (34, 47).

It is the generally accepted view that colchicine does not have any deleterious effect on the chromosomes, such as fragmentation observed following temperature treatments (21) and radiation (79). In *Rhoeo discolor* (21) a fragment of chromosome was observed,

in one microcyte but was attributed to an independent factor, since that was the only fragment ever observed by the author in material treated with colchicine. No trace of fragment was observed at the metaphase of the first division in the microspores. Eigsti (35), however, just recently has reported "chromosome breakage" in colchicine-treated *Polygonatum commutatum* pollen tubes, showing that "colchicine induces variations other than polyploid changes." Even if the colchicine effect may not be so disturbing as to cause breakage in chromosomes which can be observed but is sufficient to have a slight effect upon the chromosomes structurally, such a change in the treated material would be as important genetically as inducing polyploidy, and possibly as a method of producing mutations colchicine treatment might truly provide "multitudinous variations of a cytogenetic nature," as Eigsti has suggested.

Common among the aberrations following colchicine treatment is that of aneuploidy, which seems due to a partial instead of a total arrest of chromosome division as colchicine begins to become effective and at the time its effect is finally being eliminated. Aneuploidy may also occur from a multipolar division in some of the polyploid nuclei (52). Such aneuploidy has been recorded in *Datura* (7), resulting in $2n-1$, $4n-2$ and $4n-1-1$ types of plants. Other investigators (9, 34, 35, 52, 89) have reported $3n$, $6n$ and undetermined aneuploid individual nuclei in colchicine-affected tissues.

Chimeras, in which only a portion of the tissues is polyploid, are of frequent occurrence in colchicine-treated material. There are two types of chimeras, one sectorial ploid-chimera and the other periclinal ploid-chimera. In the sectorial type only one side of a growing bud becomes polyploid, affecting the leaves and buds of that side of the growth developed from such a bud, the other side remaining normal. This is probably the type of chimera induced in peaches (25). In periclinal ploid-chimeras epidermal layers and inner layers of cells are of different chromosomal constitution. In such a type stomata size may show the polyploid condition while pollen grains derived from inner tissue would be normal in size, and vice versa. Blakeslee and associates (9) have reported periclinal chimeras in *Datura stramonium* where, "In many cases the appearance of a given branch disagreed with the chromosome number of the generative layer estimated by size of pollen grains or determined by

actual chromosome counts in pollen-mother cells. Guard cells of the epidermis and their nuclei in some cases were of the $4n$ size, while the pollen-mother cells were $2n$. Cross sections of flower stalks in such cases showed the epidermis of the size characteristic of such $2n$ tissue. The size relations of cells have been checked by chromosome counts." They conclude: "In periclinal chimeras in which the epidermis is $4n$ and the inner tissue $2n$, it is possible to identify cells of epidermal origin by their chromosome number. It is thus possible to show that part of the internal tissue of calyx, corolla, stamens, and pistils are derived from the epidermal layer and that the chromosomal constitution of the epidermis is of major importance in determining the shape of the mature capsule."

Fertility and sterility in induced polyploids.—Breeding may consist of selfing, of crossing varieties within species, of crossing species, and occasionally of crossing closely related genera. Species of plants belonging to the same genus may have the same number of structurally similar chromosomes. Their differences may be in their respective genic constitutions. Barring incompatibility due to self sterility genes, described for *Nicotiana* by East (33), plants with such a genetic variation are ordinarily easily hybridized (17). Often, however, the differences between species belonging to the same genus may involve differences in chromosome structure (16) or in number of chromosomes, or in both. The number of the basic chromosomes in species of the same genus may vary, e.g., *Verbena* (17), $n=5$ and $n=7$; *Cornus* (15), $n=8$, $n=9$, $n=10$, $n=11$, or the numerical difference may consist of multiples of a given basic number (14, 76). If hybridization between plants varying in chromosomes structurally or numerically is at all possible, the hybrid plants will show varying degrees of pollen grain abnormality which would greatly affect self fertility. Parenthetically it should be stated that morphological abnormality of pollen grains is usually taken as the measure of sterility in plants and is determined, according to general cytological practice, by the percentage of such pollen grains present. Such pollen grains, when moistened either with water or with aceto-carmin stain, remain shriveled, and when stained either remain hyaline or stain incompletely and show abnormal contours in contrast to normal appearing pollen grains which take definite shapes by inflation and stain evenly. (There is no positive correlation between pollen grain and egg fertility (17, 18, 19), so that in

some cases where there is a complete pollen sterility, the eggs may be functional and embryos may be developed when pollen from some other source is supplied.

Fertility in a first generation plant from a cross between two given plants often depends on the amount of actual pairing of chromosomes during meiosis. This may be true if the cross is made between two diploid plants. When a cross is made between a diploid and a polyploid or between two polyploids, and their chromosomes are of homologous nature, chromosome pairing may result in partial sterility instead of fertility, which often is due to multiple instead of bivalent pairing and consequent irregular chromosome distribution during the meiotic divisions (14).

X The degree of sterility in the induced polyploids may be estimated only from the product itself. It is conceivable in certain instances, however, that the more sterile a diploid plant, the more fertile the polyploid of the same may be (11). However, it does not necessarily follow that from highly fertile plants, high sterility will result in the polyploids. In the diploids of a common variety of petunia (14) the abnormality of pollen grains ranged from 2 to 14%, while in the progeny of an apparent autotetraploid plant the abnormality ranged from 6 to 19, and in the triploids (from a cross between a diploid and the original tetraploid plant) the percentages varied from 19 to 37. (With an identical background in *Fragaria vesca* in the diploids the abnormality ranged from 1 to 17%, in colchicine induced tetraploid (24) 32 to 58, and in the triploids 46 to 61%. Here, then, we see that even in autopolyploids in some plants the sterility may be high, while in others the fertility may be high.)

The above results were derived from polyploid plants which were unquestionably of autopolyploid origin. On the other hand, referring to true allopolyploids (amphidiploids), it is generally conceded, as Darlington has suggested (11), that "the more intersterile the parent of an amphidiploid, the more fertile and true-breeding is the resulting hybrid." This may be true when hybridization is attempted between cytogenetically diverse but related species and if both plants are truly diploid plants producing monoploid gametes. Sears (81), in analyzing the induced polyploids in wheat of one intergeneric hybrid between *Triticum* and *Aegilops*, and of two interspecies hybrids of *Aegilops*, all true diploids, found that the amount of pollen fertility of the different polyploids varied in-

versely with the degree of pairing of chromosomes in the corresponding diploid hybrids. It may be assumed, therefore, that the sterility in the polyploids may be correlated with the amount of multiple pairing in some chromosomes contributed by the parent plants. Sterility in some hybrids, apparently resulting from various degrees of non-homology of chromosomes in the parents, or in hybrids resulting from crosses made between a diploid and a polyploid species, have been recently corrected by artificial polyploidy (42, 81, 86, 91). For more thorough discussion of this and related phenomena, the reader is referred to Sax's review of species hybrids (76).

TECHNIQUE OF COLCHICINE APPLICATION

The following facts and factors form some of the most important bases of colchicine polyploidy technique:

(1) Colchicine in aqueous solution is obviously diffusible into plant tissues; otherwise no internal changes could occur in meristematic cells as a result of surface application.

(2) Dormant tissues are not so affected (83) by colchicine as to result in polyploidy. Such results are obtained only in tissues where cell division is active (68). For practical results, treatments should be applied to tissues that will develop into vegetative, sexual, or both types of plant parts.

(3) It is most important to provide and maintain the best cultural conditions for the treated material in order to obtain the maximum possible results. It is especially important when material is to be immersed in colchicine to keep the solution at an optimum temperature in order that cell division may not be impeded.

(4) The duration of treatment is an important factor and should be determined for each type of material. It is dependent on the time required for the cycle of cell division in the particular tissue.

It has already been shown that the effect of colchicine is not confined to a limited number of cells at a particular stage of cellular development such as metaphase. Any cell may be affected which obtains colchicine by diffusion if such a cell goes through division while containing the chemical. Furthermore, as long as colchicine remains present in the treated material above a threshold concentration (21, 22 or 23, 47, 52, 67) the affected cells will repeatedly fail to divide at the end of each chromosomal or nuclear divisional cycle,

resulting in multipolyploidy or in multinucleate cells; consequently, successively affected tissues may often fail to survive. Undoubtedly the failure of growth and the eventual death in treated seedlings and treated growing points of shoots are often the result of the above factor. In order to avoid such an excessive result, therefore, it may be necessary to estimate approximately the optimum time limit of the divisional cycle of cells of tissues that is required for the change into polyploidy in the treated material....

- * Such an estimate was made by the writer for root tips of common onion cultured by placing the bulb on the surface of a glass of tap water. It was found that it took approximately 4 hours for root-tip cells to complete a cycle from one division to the next. Accordingly, in order to affect a group of meristematic cells in such a region in onion root-tips, these should not be subjected to colchicine effect longer than 4 hours. In onion root-tip material the time required for colchicine diffusion may be about 7 minutes (52). In 30 minutes the colchicine effect may be observed on the chromosomes at metaphase (52). Levan (52) has shown that after exposures of 7 minutes to 1 hour "there usually occurs only one *c*-mitosis [one doubling], but after long exposures several *c*-mitoses may follow each other in the same cell, and each of them doubles the chromosome number in the affected cells." He also showed that the spindle was regenerated "after a period of 12-24 hours in pure water." References to cell division rates in connection with the chemical treatment are conspicuously lacking in the literature although all experimenters give the duration of treatment, especially for material treated by immersion in the chemical solution.

The full import and the significance of cell division rates in the treated material may be appreciated from the presentation of the following data by Levan. He states: "About 48 hours after the *c*-exposure was finished, the mitoses have reassumed their normal course and persistent changes in the root cells produced by the colchicine treatment can be observed. After the short exposures (7-30 min.) such changes are rarely met with, nevertheless a few cells with $4x$ chromosomes may be found. The majority of cells show the normal diploid number. After an exposure of 1-2 hours a great percentage of $4x$ cells is found together with occasional $8x$ cells, and after the longest exposures (72 hours) cells with still greater chromosome numbers are seen, $32x$ being the upper limit in these series."

In one experiment Levan subjected onion material for 4 hours treatment to the following concentrations of colchicine: 0.0001, 0.0005, 0.001, 0.005, 0.01, 0.05, and 0.1%, and root-tips were fixed for examination 0, 8, 24 and 48 hours after the transfer to pure water. He found that "the lowest concentrations (0.0001–0.0005%) did not induce *c*-mitosis, while from 0.01% and upwards the *c*-mitoses were conspicuous." In this connection he states: "After 8 hours [after removal of colchicine] all new mitoses were normal throughout the whole series, and the spindle apparatus had completely recovered." By inference the above statement may suggest that when onion material is subjected to colchicine for 4 hours the chemical effect would extend for about 8 hours further; therefore, it may require that many hours to eliminate colchicine effect in the material exposed to 0.1% and lower concentrations by keeping the material in water for a duration of 8 hours, and it should require longer washing if higher concentrations of the chemical than the above are used. It is apparent, therefore, that if the cell division rate in such material is 4 hours, and in about 7 minutes cells can become affected, then during a 12-hour period (4 hours in the solution and 8 hours in water) some cells would have gone through two or three divisions and chromosome doubling would have occurred that many times, assuming that colchicine had no retarding effect on the division rate (it has been shown by some authors that colchicine did have such an effect). However, Shimamura (83) has shown that in the onion "the effect of colchicine on the cells differs even in the same tissue of the same root-tips, depending first upon the different stages of nuclear division, and second whether affected cells belong to tissue that is growing vigorously or one that is dormant." Thus the root treated with colchicine becomes of "mixoploid" nature. He further finds that "when mitosis has resumed its normal course, most of the changed cells are found in the older parts of the root, while normal diploid numbers are found close to the root cap among new meristematic cells." Both Levan (52) and Shimamura (83) have indicated that in the treated root material diploid cells may predominate in competition with the polyploid cells. Therefore, the new growth in the root-tip in the root-cap region evidently may continue as diploid tissue.

The results with lilies (36) that were treated by immersing the vegetative tip of each individual plant in colchicine solution probably

indicate that, as in root tips, the real end of a vegetative branch may grow as a normal diploid, the polyploidy effect being confined largely to the lateral growth. It may be desirable, therefore, following the vegetative tip treatments to encourage lateral shoot development rather than to remove laterals and encourage the growth in the tip. The polyploidy in lateral growth could result from colchicine diffusion into the lateral buds. In some material the treated tips may die following the treatment, especially when immersed in solution for a considerable length of time. In such cases polyploidy could have been induced in the lateral buds in the proximity of the injured portion; therefore, growth in these regions should be encouraged. Both Dr. S. L. Emsweller at this station and the writer have had evidence in support of the above suggestions.

(5) Concentration of the chemical is an important item in considering the method of treating organisms as long as it does not fall below an effective minimum, a threshold point which is determinable with each treated material. With some animal material Ludford (57) found the threshold concentration as low as 0.00001%. In the fertilized eggs of *Arbacia* the effective point was found to be just above 0.00112% (64), and for the fertilized eggs of rabbit the effective percentage of solution was 0.000041% (70). In plants, for stamen hair cells, the threshold was just above 0.004% (64); for root-tip cells of onion, 0.0075% (52); and for vetch root-tips Koltzoff (47) found that colchicine even at 0.0006% was effective. The writer germinated seeds of *Trifolium hybridum* L. (Alsike clover) on filter paper moistened with distilled water in a Petri dish. Of the young seedlings equal numbers were transferred to separate Petri dishes with filter paper. One of these was moistened with 25 drops of distilled water and others with the same number of drops of colchicine in the following percentages: 0.01, 0.025, 0.05, 0.1, 0.2, and 0.5. The duration of colchicine cultures was 8 hours, after which time they were rinsed with distilled water for one-half hour and then transferred to separate distilled-water cultures. The cultures with distilled water and colchicine at 0.01% grew evenly, while cultures with 0.025% and above were stunted uniformly and remained that way for over 1 week, and then were discarded. The seeds of this species germinated in less than 24 hours, which fact makes this material very useful in demonstrating or testing colchicine effect. Seeds of *Rhoeo discolor* were germinated similarly;

they germinated in from 17 to 33 days. Four experiments were conducted in each, using 1- to 3-day-old seedlings. Colchicine treatment was done by shallow immersion of the material in the solution. After soaking, the seedlings were rinsed in water for one-half hour. (a) 16 seedlings were treated with 0.2% colchicine for 6 hours; of this number 8 grew to maturity, of which 1 was apparently wholly tetraploid (determination by pollen grain size and by counts of chromosomes in the microspores at first division). (b) 4 seedlings were treated with 0.5% for 6 hours; all 4 grew to maturity and none were tetraploid. (c) 14 seedlings were treated with 0.2% for 22 hours; only 3 seedlings grew to maturity, while the rest died without making much growth after they were planted; of these, 1 was apparently wholly tetraploid (determination as above). (d) 14 seedlings were treated with 0.5% for 22 hours; only 1 seedling grew at all, and it grew very slowly; it was sectorially affected, one-half being diploid and the other half tetraploid. When strawberry seeds (*Fragaria vesca*) (24) were germinated in colchicine and were planted immediately after germination, none grew beyond cotyledon expansion. In another experiment very young seedlings were treated for 24 hours; of these only 1 survived, which grew into a tetraploid plant, while others remained at cotyledon stage and died eventually.

The above experiments seem to indicate that there is a high mortality of seedling material if the treatments are extended over a long time. The total exposure of the seedlings to colchicine could account for a certain percentage of the mortality. Lutkov (58), on the other hand, treated only the stem side of flax seedlings while the roots were covered with filter paper moistened with water. His tabulated results show that the mortality of seedlings is increased with the duration of the treatments, especially when the treatments extended to 48 hours, while the percentage of polyploids against the total number of seedlings treated was practically the same. However, the total number of polyploids from the treatments of short duration (6, 12, 18, 24, and 36 hours) was higher than from long duration (48 hours). Apparently, in flax 36 hours of treatment was not very harmful, perhaps because of the method of treatment. In two instances with 24-hour treatment, and in one instance with 36-hour treatment, out of 51 seedlings treated 41 survived; of these 10 were octoploids, 23 were tetraploids, and the remaining 8 were

diploids. Instances of octoploidy resulting directly from colchicine treatments of diploid plants have been very rare (8); therefore, such a high proportion of octoploidy in flax seems very remarkable. On the other hand, generally the lack of octoploids from treated diploids ought to be considered rather noteworthy, since cytological observations on the experimental material have demonstrated often that higher polyploidy than tetraploidy is prevalent. The discrepancy here referred to is perhaps due to a difference in the rate of development and growth between tetraploid and octoploid cells and tissues, or is due to probable abnormal nature of higher polyploid nuclei (22).

Outline of methods. The technique in colchicine applications to induce polyploidy involves four items:

(1) Types of material to be treated: seeds; seedlings; growing tips of twigs or expanding buds, or bulb scales (Emsweller and Lumsden, unpublished).

(2) The media in which colchicine may be applied: aqueous solution; weak alcohol; a suitable emulsion; lanolin paste; agar solution; glycerine and water, or glycerine and alcohol, with or without a spreading reagent.

(3) The range of concentrations of the chemical used: the lowest perhaps 0.0006% and the highest about 1%.

(4) The duration of treatments: merely wetting to over 24 hours.

The following are brief outlines of methods based on the type of material treated. These methods may be modified to suit the material and inclinations of each experimenter.

1. Seed treatment. Seeds of *Datura*, *Cosmos*, *Portulaca* (7) and *Nicotiana* (86, 91) have been soaked in 0.2 to 1.6% aqueous solution of colchicine for 4 to 10 days. By seed treatments a large number of polyploid plants have been obtained. This method may very well apply to seeds that are normally germinated in a few days, and such seeds may be planted before germination. Pal and Rammanujam (69) obtained very noteworthy results by treating seeds of chili (*Capsicum annum*). Seeds were immersed in 0.05, 0.1, 0.2, and 0.4% colchicine for 1, 2, 4, 6 and 8 days, respectively. It is interesting to notice that seeds treated for 1 day in 0.05 and 0.1% produced 7 and 5% tetraploids, respectively, while treatments with 0.2 and 0.4% gave 73 and 60% tetraploids. Mortality in seeds

appears to be high both in strong solutions and in treatments of long duration. The writer (24) had complete failure in treating *Fragaria vesca* seeds with almost the same technique as Pal and Ramamujam. Simonet and Chopinet (84) do not appear to be enthusiastic about the seed-treatment method, for the treating of thousands of seeds had resulted in failure. However, if seed treatment and seed germination can be timed well, there is no reason why consistent success cannot be obtained with suitable material. In some cases where seeds naturally germinate very long after planting, they should be treated either at the time they begin to germinate or immediately after germination.

2. Seedling treatment. Freshly germinated seedlings are immersed in colchicine solution in a shallow container, or placed on filter paper thoroughly wetted with the solution, for 3 to 24 hours, depending on the kind of seedling or the rapidity of growth. It was found, for example, that for the young seedlings of clover 8 hours of treatment was too long. Treating the stem side of seedlings alone, instead of total immersion, may give excellent results (58). However, the result in polyploidy production will depend on the active cell divisions in the epicotyl region of the seedlings treated.

The writer was able to induce polyploidy in *Cosmos* by moistening the soil over and around the seedlings with 0.02 to 0.1% aqueous solution, just at the time the seedlings were pushing the soil up. Following this procedure, clover and timothy seedlings grown in flower pots were similarly treated. By visual comparison the treated seedlings appeared affected. A number of the treated timothy plants developed a dwarf habit of growth, though no cytological examinations have as yet been made. This method of treatment may have a number of advantages over that of treating entirely exposed seedlings that are either germinated on filter paper or removed from the soil for treatment, as seedlings are not disturbed by this method and there is less mortality following the treatments.

3. Treating growing shoots and buds. Tips of rapidly growing shoots and newly expanding vegetative buds of woody as well as herbaceous plants, and tips of young seedlings may be treated by brushing the colchicine solution over partially exposed tips once or several times, or by immersing such material in a suitable vessel or small vial containing colchicine solution for a few hours (36), a day (5) or 2 days (73), depending primarily on rapidity of growth of

the treated material. A mixture of 0.5 to 1.0% colchicine in lanolin (8, 37, 62) may be smeared on the growing portions of young shoots and on the expanding branch buds. The application of tepid 1% colchicine-agar solution (1 part 2% colchicine and 1 part 3% agar) by brushing over the growing tip of young seedlings of petunia (54), and colza and flax (84) has given very good results.

Warmke and Blakeslee (91) have recommended an emulsion to replace the use of lanolin alone, as a base for colchicine, as the use of lanolin has a number of inconveniences. The formula is as follows:

Stearic acid	1.5 gm.
Morpholine	0.53 cc.
Tap water	20.00 cc.
Lanolin	8.00 gm.

The details of preparation are given in their paper. This emulsion is recommended as a spray, but it seems questionable that spraying of colchicine should be recommended, since it may be easily applied in many ways less hazardous to the eyes and skin of workers.

Nebel (66) has used 5% alcohol as a medium for colchicine in treating tips, probably for the purpose of improving penetration into the treated regions.

In treating growing tips and very early stages of expanding branch buds, the writer has used colchicine in a viscous medium in order to keep the treated portion moist with the colchicine solution for about 24 hours, and thus possibly to maintain a supply of colchicine in solution at the treated region and extend its effect to wider areas in the growing tissues. Also an attempt has been made to develop such a viscous medium capable of penetrating into the folds of young leaves and between protective hairs thus carrying with it a supply of colchicine to growing primordial regions that are covered with scales and young leaves or petiole sheets.

Olive oil and water, and paraffin oil and water were used, to both of which a commercially prepared emulsifying and wetting agent, Santomerse,¹ was added. An emulsifying hand machine was used to emulsify the mixture. Both these emulsions, alone or with

¹ "Santomerse" is a chemical preparation used as an emulsifying agent and prepared by the DuPont Co. Its use was recommended to the writer by Dr. M. C. Goldsworthy and Mr. E. L. Green of this Station.

enough colchicine to make a mixture of 0.16%, were used in treating the central portion of young strawberry plants. Both oils were found injurious to the plant and caused leaf curling. They also caused much more retardation of growth of the plants than that produced by colchicine alone. However, when the emulsions containing colchicine had been used, some of the lateral growths developed into polyploids, while the central growth usually grew normally.

Instead of using either olive oil or paraffin oil for colchicine, we are now using glycerine-water or glycerine-alcohol (5 or 10%), adding to either a few drops of Santomerse. It is applied with either a small brush or medicine dropper. The proportions are as follows:

Glycerine	7.5 cc.
Water	2.5 cc.
10% Santomerse	6 to 8 drops

or

Glycerine	7.5 cc.
40 or 20% alcohol	2.5 cc.
10% Santomerse	3 to 8 drops.

Either colchicine powder is added to the above solutions, or colchicine in water or alcohol solution is mixed with glycerine. The concentration of colchicine in this mixture may be varied from 0.1 to 1.0%: weak concentrations for fast growing tips, strong concentrations for slow expanding buds. The proportion of glycerine may be changed to either strong or weak, depending on how viscous or how liquid a solution is desired; a stronger glycerine solution may cause more injury than a weak one. In order to prevent glycerine injury during sunny days the treated material should be shaded. If desired, after a certain time, the glycerine mixture may be washed off with water so that possible excessive colchicine effect may be prevented, and to lessen glycerine injury. Glycerine possesses the desired viscosity, and it is soluble in both water and alcohol; however, it possesses a high degree of surface tension, which is in some degree lowered with alcohol and to a greater extent with Santomerse. Colchicine is soluble in these solutions, while it is not soluble in the oils. Glycerine helps to keep the colchicine solution at the treated portion for at least 24 hours, which feature is one of the most desired conditions in the application of colchicine, especially when it is applied to slow growing material. }

DETECTION AND DETERMINATION OF POLYPLOIDY

Polyploidy, being defined as chromosome doubling, is best determined by chromosome counts. Such determination is greatly facilitated by speedy methods, and must still be used as a final method of examining plants that are believed to be polyploid.

There are a number of characteristics that generally express polyploidy and are usually associated with it. One major characteristic involves change of size; another, change in shape (85) of plant parts, such as leaves, fruits, *etc.* There are measurable changes that are used in selecting colchicine-affected plants or parts of plants from those unaffected.

There is ordinarily a correlation between nuclear volume and cell volume. Thus, if the volume of the nucleus is changed following chromosome doubling the volume of the cell would also change. Often with the change of volume a change in the dimensions of cells occurs, which with a change in size may become apparent in some plant parts, such as leaf, flower, fruit, seeds, *etc.* The measurements of either or both stomata and pollen grains are used by research workers in further detecting polyploidy. In cases where experiments are performed with sterile plants, any change in sterility is also an indication that polyploidy has occurred. While these gross changes may be used to detect probable polyploidy in experimental material, actual chromosome examination and counts are necessary to determine the nature of such material.

There have been some exceptional cases where polyploidy could not be detected either by external structural changes or even by measurements of stomata. Such a condition was encountered in the induced polyploid material of wheat crosses by Sears (81). The polyploidy in a small part of this hybrid was detected by the occurrence of fertility which was absent from the diploid hybrid. A similar instance was reported by Lapin (51) in a species hybrid of basil (*Ocimum*). The measurements of pollen grains indicated 45% increase in size. It is apparently a universal phenomenon that following a doubling of chromosome number there is always an increase in the pollen grain size. Thus the detection of *induced* polyploidy may be more accurately made through measurement of pollen grains than of stomata. One should be warned that even this method cannot be safely used in detecting polyploidy in natural or cultivated varieties and species. The writer has found pollen grain size to be

very uniform in peach varieties (20); however, it varies so much in grape varieties (unpublished data) that some diploid varieties produce pollen grains as large as one tetraploid variety, Eaton.² The tetraploidy of Eaton was detected by this method, but its polyploidy had to be confirmed by chromosome counts of root tips from cuttings. It may be safe to assume that the uniformity of pollen-grain size in the peaches is due to the fact that the varieties belong to one single species of the genus *Prunus*, namely, *P. persica*, while the varieties of grapes are from a conglomeration of species of the genus *Vitis*. Significantly, the name peach ordinarily stands for one species, *P. persica*, whereas the name grape stands for the whole genus *Vitis*; hence the difficulty referred to above. Similar difficulties are encountered when stomatal measurements are used as a method of detecting natural polyploids (75, 80).

THE NATURE OF SOME OF THE CHANGES FOLLOWING INDUCED POLYPLOIDY

The most observable consequences of induced polyploidy are usually increase in size and shape of plant parts: leaves, stature of plants, branches, flower parts, fruits and seeds. These changes, as stated above, do not always follow polyploidy. Therefore it may be assumed that such changes are effected, not alone by volumetric change in the cells following an increase of chromatin content, but also by a genetic factor or factors inherent in certain forms. Such factors apparently can suppress changes in size as well as in shape (4, 51, 81). In two seedlings of peach, polyploid sectors could not be distinguished from a normal sector by either size or shape of leaves, but were detected through stomata measurements, the stomata being much larger in the tetraploid sectors. Branches were isolated from the probable tetraploid sector and are now propagated. This would be the first instance of tetraploidy occurring in peaches (25); therefore the study of sexual phases of these sectors would be of considerable interest besides other considerations of physiological as well as of economic nature.

The chromosome duplication may result in one of at least three types of tetraploids: (1) There may occur an appreciable increase in size of each vegetative cell in the tetraploid individual, while the total number of cells making up the plant remains relatively the

² The assistance of Mr. I. W. Dix of this station in the detection of polyploidy of this grape variety is here acknowledged.

same as in the diploid form; consequently the tetraploid plant appears larger than the diploid individual. Most of the changes following polyploidy appear to fall into this category. (2) An increase in cell volume may follow a doubling of chromosomes, but there may be a decrease in the total number of cells making up the tetraploid plant; therefore the tetraploid individual will not appear different from the diploid (4). (3) The doubling of chromosomes may not have any effect on the size of the cells. The polyploid individual remains indistinguishable, except probably in sexual (51, 81) and in some obscure physiological behavior.

Besides structural and other typical quantitative changes referred to above, there also appear to be some changes, such as in the intensity of color, fragrance, and other characters, which may be called qualitative, although when scrutinized more closely most of them may be of a quantitative nature. Kostoff (48) found proportionate increase in the intensity of the green color of leaves in tetraploids and octoploids. He found that this change was associated with increased depth of leaf tissue in the polyploids, and with a proportionate increase in the number of chloroplasts. Incidentally he did not find any appreciable change in size of the chloroplasts in the three types of plants ($2n$, $4n$ and $8n$). He found, however, the extracts of chlorophyll from $8n$ most concentrated, and in $4n$ more than in $2n$. The writer has observed progressive intensity of green color in the leaves of successive autopolyploid *Fragaria vesca* plants.

Morrison (60) found intensification of flower color and also of fragrance in Marigold flowers following chromosome doubling. Ruttle and Nebel (74) failed to find fragrance change in the variety of Marigold with which they were working. They, however, have reported a change of odor in the tetraploid spearmint (*Mentha aquatica* \times *M. rotundifolia*), which differed from the diploid. They have further reported a change in the oil "possibly in both quantity and quality . . ." in the tetraploid *Ocimum basilicum* L. Lapin (51) did not find any increase in fragrance in the induced amphidiploid obtained from the hybrid of *Ocimum canum* Sims. \times *O. gratissimum*. On the other hand, Golubinskij (43) has spoken of "unbearably strong" odor of camphor in a tetraploid plant form that was isolated from adventitious shoots, produced from callus tissue of diploid *O. canum*.

The work of Kostoff and Tiber (50) with rubber-producing Koksaghyz variety of *Taraxacum* is suggestive of how polyploidy work may be directed toward definite purposes, the result of which may be of some value. From young seedlings treated with 0.25% colchicine for 20 hours, they were able to isolate one that later developed thicker leaves, had larger pollen grains, and produced larger seeds than the diploid. They have as yet no records as to the amount of rubber in the new form:

- Kostoff and Aksamitnaia (49) in 1935, before the present "era" of colchicine polyploidy, made an interesting comparative analysis of autotetraploid and diploid tomatoes and reported the following relationship. The tetraploid had higher water content per unit of tissue, more nitrogen and proteins, and "more carbohydrates before the hydrolysis"; while the diploid had more ash and more starch.

Randolph and Hand (72) in 1938 made a very interesting analysis of carotinoid content in both diploid and tetraploid yellow corn. In the tetraploid they found 43% increase of carotinoid and "the same percentage increase in vitamin A activity, since both the active carotinoids, beta carotin and cryptoxanthin, and the inactive zeaxanthin were increased to the same extent." They have presented the following table, which gives cell volume, gene and carotinoid relationship in the endosperm cells of the diploid and tetraploid:

	<i>Diploid</i>	<i>Tetraploid</i>
Cell volume	1	3.5
Carotinoid per unit volume	1	1.43
Carotinoid per cell	1	5
Genes per cell	1	2
Genes per unit volume	1.75	1
Carotinoid per gene	1	2.5

The data show that cell and nuclear volume relationship is not the same and may change with different material, and it is perhaps subject to genetic factors (14, 17), as was pointed out above. In the above table the most striking reverses in relationship are found in the increase of endosperm cell volume, which is 3.5 times more in the tetraploid than in the diploid. Respectively, there is an increase of 5 times in carotinoid content per cell and 2.5 times carotinoid per gene, while gene per unit of volume in the tetraploid is less compared with the diploid. The observed differences between diploid and tetraploid yellow corn are attributed "to quantitative rather than qualitative gene differences, since," it is stated, "the compari-

son was made between strains having a common origin and an essentially identical genetic constitution."

Sullivan and Myers (87) studied the chemical composition of diploid and tetraploid *Lolium perenne* L. They found that "the tetraploid plants were higher than diploid in reducing sugars, sucrose, total sugars, and in the proportion of dry matter that was soluble in 80% alcohol." They point out, on the other hand, that "the tetraploids . . . were, in general, lower in both soluble and insoluble nitrogen, but the differences were of such slight magnitude as to have no statistical significance. No consistencies were found in total dry matter and crude fiber." Thus they remark, "It may be concluded that under the conditions of the experiment and with the material used, chromosomal and genic reduplication causes an increase in the sugar content of *Lolium perenne*."

It is worth noting that in the tetraploid tomato Kostoff and Aksamitnaia found more nitrogen and proteins, while Sullivan and Myers found that in *Lolium perenne* tetraploids the nitrogen content was not significantly different from that of the diploids. Also, no significant difference was found in percentage of dry matter and crude fiber between the samples of diploid and tetraploid plants of *L. perenne*; while the percentage of alcohol-soluble dry matter was higher in the tetraploids of *L. perenne* than in the diploid. On the other hand, tetraploids of *L. perenne* and tomato contained more sugars than diploids.

In consequence of doubling of chromosome number, the cells, as pointed out above, may or may not increase in volume; however, obviously in either case there is a chromosome increase, at least in number if not in volume. It is conceivable that changes following polyploidy would result in changes of relationship between a number of physiological factors which would be expressed in the polyploid plants.

It is probable that whenever a change in size of cells occurs following polyploidy, it will affect some changes in the functioning of the protoplasm of the cell as a whole, since the original relationship between cell volume and surface becomes altered, because the surface of a body does not increase at the same mathematical rate as the volume. The change of volume and surface would also affect the depth of the cells. Similarly, it is probable that changes of relationship would occur between total chromosome volume and total chro-

mosome surface, nuclear volume and nuclear surface, and between these and cytoplasmic volume and cell surface. It may therefore be assumed that measurable differences between a diploid and, especially, an autopolyploid vegetative sib are expressions of a physiological nature resulting from physical changes of relationship referred to above, rather than of a genetic nature resulting from numerical duplication of genetic factors, *e.g.*, genes. It is improbable that the mere duplication of all genes would cause an alteration of the balance between the genes which was present in the diploid. This may be compared with a state of balance existing between chemicals in a solution. The chemical balance would remain unchanged when the volume of the solution is doubled, while the quantitative relationship which previously existed between the chemical elements is maintained.

However, a change in the balance of genes—their activity and genetic expressions—would perhaps result from some physiological consequences of polyploidy which may be in the nature of changes in oxidation and respiration rate; viscosity movement of protoplasm, including cytoplasm and nucleus; in the rate of intake of various substances and output of various metabolic by-products; in enzymatic activities; and in a number of other physiological phenomena. If any of these changes occurred following the doubling of chromosome number, it undoubtedly would effect some changes in the polyploid plant, which may be measurable, as was recorded in a few instances here referred to.

Undeniably it would be of extreme importance if experiments could be devised to find out precisely what are the factors involved in changes following polyploidy. With the present methods of treating seedlings, vegetative tips, or bulb scales (Emsweller and Lumsden, unpublished), or any vegetatively propagated material, we obtain sectorial or entire polyploids. This gives us material of known source which could be used in measuring polyploidy changes and compared with its vegetative diploid counterpart. With such ideal material, carefully devised physiological studies might lead to our understanding of how, for example, a polyploid plant as compared with its vegetative diploid sib ~~etc.~~ be more or less hardy in a given environment; how, if at all, plant parts and their products—fruits, nut products, cereals, vegetables, oil products of various sorts, *etc.*—may be modified quantitatively as well as qualitatively; how, if at

all, chemical products such as vitamins and other important substances may be increased in numerous economic plants. The results of these analytical experiments would assist in the purposeful use of polyploidy, which can now be quite readily induced in plants, through the use of colchicine and similarly effective agents. ✓

NOTE.—Literature relevant to the subject presented above is given in two lists. The first list refers to literature cited in the text and the second to literature which, although it was not reviewed here, contains valuable information to which the reader could refer, as indicated in the titles.

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THE MICROBIOLOGY OF CELLULOSE DECOMPOSITION AND SOME ECONOMIC PROBLEMS INVOLVED¹

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INTRODUCTION

True cellulose is characterized by well defined botanical and chemical properties and by a characteristic crystal structure. Although resistant to ordinary chemical reagents and digestive juices of higher animals, it is readily decomposed by a great variety of microorganisms, as a result of which it does not tend to accumulate in nature. In many early studies on digestion of cellulose by microorganisms, insufficient differentiation was made between true cellulose, on the one hand, and other carbohydrates comprising starches, hemicelluloses and polyuronides, on the other. Frequently even compound celluloses, containing lignins, pectins and other incrusting substances, were not distinguished from cellulose itself.

Among the structural constituents of cell walls of plants, cellulose occupies a prominent place. Upon the death of plants, whether by natural agencies or through injury caused by animals, cellulose is attacked by a great number of different microorganisms, with the result that it tends to disappear very rapidly. The nature of these organisms, as well as the chemical processes that they bring about in decomposition of the cellulose, varies considerably, depending upon environmental conditions. Included among the organisms are numerous bacteria, fungi, actinomycetes, protozoa and possibly certain insects and a variety of other invertebrate animals possessing distinct morphological and physiological characteristics.

Some microorganisms capable of decomposing cellulose, notably

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certain bacteria, are highly specialized and depend upon cellulose as their exclusive source of energy. Others, including some bacteria and all the fungi and actinomycetes, depend only partly upon cellulose as their nutrient. Frequently, several organisms collaborate in the disintegration of the cellulose molecule. The products of decomposition of cellulose by one organism may become sources of energy for the activities of other organisms. Cellulose is digested by a variety of animals, ranging from a number of insects and worms to higher, herbivorous and omnivorous animals. This process is carried out, at least in higher animals and insects, through the associative action of the animal with different microorganisms inhabiting its digestive tract. Finally, some cellulose may become fossilized or rendered physically resistant to microbial attack; this can account for only a mere trace of the total cellulose synthesized by plant life on the surface of the globe.

CLASSIFICATION OF CELLULOSE-DECOMPOSING ORGANISMS

A number of different systems have been proposed for classification of cellulose-decomposing microorganisms, based primarily upon morphology and taxonomy. A more logical system, however, appears to take into consideration the substrate in which cellulose decomposition takes place, as well as the conditions of its decomposition. A system of this nature may be outlined here briefly as follows:

1. Microorganisms concerned in cellulose decomposition in growing plants, comprising a number of plant pathogens, primarily among the lower or filamentous fungi and the higher or fleshy fungi.

2. Microorganisms assisting in the decomposition of cellulose in the digestive tracts of insects and higher animals; these may be considered primarily as symbiotic organisms, and comprise certain anaerobic bacteria and protozoa; aerobic bacteria may also participate.

3. Microorganisms bringing about the digestion of cellulose in purification of sewage and in disposal of garbage; the first comprise various aerobic and anaerobic bacteria, and to a less extent actinomycetes and lower fungi; in the second process, a number of fungi and bacteria, including thermophilic groups, are largely concerned.

4. Microorganisms concerned in cellulose decomposition in soils and in composts; a great many fungi, bacteria, actinomycetes and possibly also various invertebrate animals are responsible for these processes.

5. Microorganisms active in cellulose decomposition in peat bogs, leading to the formation of various peats and coal; anaerobic bacteria and some aerobic forms are active under these conditions.

6. Microorganisms bringing about decomposition of cellulose in oceans, rivers and other flowing water systems; these comprise mostly aerobic bacteria.

7. Microorganisms decomposing cellulose in timbers, paper pulp, textiles, manufactured paper and books; a great variety of lower and higher fungi, actinomycetes and aerobic bacteria as well as certain invertebrate animals, such as termites and shipworms.

8. Microorganisms utilized in fermentation of cellulose in certain industries, as well as in the preparation of certain foodstuffs; here belong anaerobic bacteria and a number of fungi.

TYPES OF CELLULOSE DECOMPOSITION

The decomposition of cellulose in these different substrates and under various conditions is thus found to involve a great number of organisms, a knowledge of which is essential before their activities can be understood and methods of control and utilization efficiently developed.

Plant pathogens. Destruction of cellulose in the healthy plant first attracted the attention of plant pathologists during the latter part of the last century. Because insufficient distinction was made between the chemical nature of various hemicelluloses and that of true cellulose, cellulose decomposition was not well understood until the early part of this century.

Soon after the beginning of the present century, it became recognized that at least three groups of organisms are capable of bringing about destruction of cellulose in nature, namely, anaerobic bacteria (72), aerobic bacteria (110) and fungi (68, 7, 56). These early investigations were soon followed by numerous contributions in which the role of bacteria was at first considerably confused and aroused much discussion, whereas the rôle of fungi was not sufficiently appreciated. Only in very recent years has it become

definitely established that the nature of the microorganisms decomposing cellulose varies with the nature of the substrate and the conditions of decomposition.

It was also found that actinomycetes (57), including a variety of forms, as well as numerous higher or fleshy fungi, play an important part in cellulose decomposition. The last group is of particular importance in the destruction of cellulose in growing trees and in timbers. *Fusaria* (1, 91) and various other fungi were shown to be capable of destroying cellulose in herbaceous as well as in other annual and perennial plants. The filamentous fungi acting upon cellulose in soils and in composts were shown to include, in addition to species of *Fusarium*, also *Aspergillus*, *Penicillium*, *Trichoderma*, *Chaetomium*, *Humicola*, *Cephalosporium*, and others. The cellulose-decomposing bacteria were found to comprise many morphologically and physiologically distinct groups.

In the breakdown of wood by fungi, three stages have been distinguished (92, 42): (a) attack by various Uredineae and Ustilagineae which decompose the sugars and starches; (b) invasion by saprophytic fungi belonging to the genera *Mucor*, *Penicillium* and *Aspergillus*, resulting in complete destruction of the simple carbohydrates and starches and also in decomposition of hemicelluloses and polyuronides; (c) the entrance of true wood-destroying fungi, belonging to the Polyporaceae and Agaricaceae, which also attack the cellulose and lignin.

Among the last group of organisms, two sub-groups have been recognized (27): (a) those that attack cellulose in preference to lignin (brown rots); (b) those that decompose both lignin and cellulose, frequently the first somewhat more rapidly than the second (white rots), which may lead to cellulose enrichment in the residual material (88, 11). A number of higher fungi, including the genera *Boletus*, *Comiophora*, *Stereum*, *Fomes*, *Lenzites*, *Merulius*, *Russula*, *Psalliota*, *Polyporus*, *Trametes*, *Armillaria* and *Coprinus*, were shown to be capable of decomposing cellulose in wood; some of these specialize on certain trees, while others attack timbers or grow on the cellulose in composts. Various insects may also be concerned in wood destruction although, in some cases at least, these insects are known to be assisted by cellulose-decomposing bacteria (126) or possibly even by protozoa (17).

Animal systems. The various animals which digest cellulose

can be conveniently divided into four groups: (a) ruminant animals; (b) omnivorous animals, including *Homo sapiens*; (c) wood-boring insects; (d) worms, snails and other invertebrates. In spite of the accumulated evidence concerning the utilization of cellulose by higher animals, the nature of the process thus brought about is still imperfectly understood. This is due, at least partly, to improper methods of cellulose determination in foodstuff analysis, whereby this compound is usually included, together with lignin and certain other chemical substances, in the "crude fiber" fraction. Another, perhaps more important, reason is found in the difficulty of isolating the specific bacteria concerned in the digestion of cellulose in the tracts of animals, in order to measure their activities, and to elucidate the relation between the function of the animal and of the microorganism in the digestion process. The fact that cellulose is as efficient a source of energy for the animal as is starch or other digestible carbohydrates led to the assumption (53) that cellulose is also utilized by the animal, without the agency of bacteria. As far back as 1882, however, Tappeiner (102) concluded that bacteria are responsible for the digestion of the cellulose by herbivorous animals. Later investigations (94, 40) brought out the fact that certain specific bacteria, notably anaerobic forms, are primarily concerned in the process. The chief seat of activity of these bacteria, in ruminant animals, is the rumen. The bacteria convert the cellulose to various intermediary products, notably dextrins, sugars, organic acids and alcohols, which are utilized directly and completely by the animal. The bacteria are thus able to consume only a small part of the energy in the breakdown of the cellulose. Aerobic cellulose-decomposing bacteria, belonging to *Cytophaga* and *Cellvibrio* groups, have also been demonstrated (2) in the rumen and caecum of herbivorous animals. Digestion of cellulose in the caecum of higher animals is also brought about by an indigenous microflora and microfauna (4). This microbiological population was found to be intermediate in its characteristics between that of the human mouth and that of river mud.

Wood-boring insects, comprising termites, cockroaches and numerous others, have been shown to be definitely assisted in their digestion of cellulose by microorganisms. Among these, protozoa are claimed to be of special significance (17). The relationship

between termites and protozoa is said (43) to be symbiotic, the former comminuting the wood and transporting it to the hind-gut, and the latter digesting it anaerobically; some of the metabolic products are then absorbed by the hosts.

The shipworm, *Teredo navalis*, lives in the wood of ships partly submerged in salt water. It bores through the wood, passing the borings through its digestive system; a large part of the hemicelluloses and cellulose is consumed by the organism, leaving the lignins practically intact (22). To what extent bacteria are responsible for cellulose digestion by these molluscs, similar to that of the action of bacteria in the digestive tract of herbivorous animals, still remains to be determined. It is said that the organism produces an enzyme, cellulase, which enables it to digest the cellulose. Termites, or "white ants," also extract their nutrients from wood and use the undigested residue for the building of their nests. Oshima (73) has shown that as a result of this decomposition the cellulose was reduced from 54.6 to 18.0% and the pentosans from 18.0 to 8.5%. An enzyme which acts upon cellulose has been demonstrated beyond doubt (51) in certain animals, notably snails.

Sewage and garbage. A large part of man's food consists of cellulose, notably the roughage, in the vegetable constituents of the diet. This cellulose is only partly utilized in the digestive system and is largely excreted in the faeces. The life of man, especially the city dweller, involves the handling of a variety of cellulosic materials, ranging from the paper that he reads and the bags and wrappers in which he buys his clothing and foodstuffs to the kitchen residues left in the preparation of his meals. These find their way sooner or later into sewage and garbage. Disposal of these waste products presents to city dwellers, especially in thickly industrialized areas, important problems in sanitation. This involves destruction of human wastes by agencies which are least expensive, least offensive, and which may result in recovery from the wastes of the valuable elements nitrogen, phosphorus and potash which can be utilized for agricultural purposes. The activities of microorganisms fulfill these requirements. In sewage disposal systems, bacteria of the aerobic and anaerobic types are chiefly concerned. The anaerobic organisms produce combustible gases (H_2 , CH_4) which become valuable by-products, because of their fuel value; the nature of the acids and alcohols formed is still unknown, and they remain, therefore, unexploited.

During digestion of solids in sewage, cellulose was found (37) to decompose rapidly; as much as 79% of it disappeared in three weeks; addition of lime hastened the process, increasing decomposition to 96% in the same period of time. A large part of the products of cellulose digestion is liberated as gases. The residual sludge contains a higher percentage of carbon than the original sewage solids, since it is very low in cellulose and is enriched in lignin and its transformation products.

Disposal of garbage involves the activities of numerous microorganisms. Composting is the most convenient manner of disposing of house wastes, if they are not to be burned and most of their fertilizing value thereby lost. Various processes have been developed for utilizing these waste materials (40, 31). The fertilizing value of the residual matter was found to be very high, as compared with farmyard manure and with artificial fertilizers (83). Without going into a detailed discussion of the problems of composting house wastes, it is sufficient to emphasize that the underlying principles are the same as in the case of farm wastes. Favorable aeration, moisture and reaction are essential for the most rapid and economic processes. These consist in destruction of the cellulose and hemicelluloses, and in preservation of the nitrogen and other nutrient elements. Fungi and bacteria, frequently thermophilic forms, are usually concerned in the transformations. In some cases, certain actinomycetes also may play an important part.

Soil and composts. The major part of the cellulose synthesized by plants is left on the land or in the forest as waste material, in the form of roots, stubble, straw and other farm residues. These wastes are destroyed by microorganisms either directly, in the soil and in the compost, or after they have first passed through the digestive systems of animals; the animal manures, consisting of animal excreta and bedding, high in cellulose, also find their way into the soil directly or after they have been allowed partly to decompose in the compost heap. Both in soils and in composts, cellulose is readily destroyed, giving rise to a product which is designated as humus. This humus plays an important rôle in soil fertility and is highly significant in soil conservation. The contribution of cellulose to the formation of this humus is indirect, that is, through the synthesizing activities of the microorganisms. The numerous fungi, actinomycetes, aerobic and anaerobic bacteria, as

well as myxobacteria (67), which bring about destruction of the cellulose in field and garden soils, in forest litter and in composts, synthesize extensive quantities of cell substance which forms a major contributing factor to the formation of humus. In this process, large amounts of nitrogen, phosphorus and certain other inorganic elements are removed from circulation and are incorporated in the organic constituents of the humus. The problem of successful farm management, application of inorganic fertilizers, use of green manures and utilization of stable manures and of composts are thus controlled by the various processes of cellulose decomposition. An extensive literature has accumulated concerning the organisms involved (118, 119). Their specific nature depends upon the particular soil conditions. Although available nitrogen is essential for the breakdown of cellulose, the possibility is not excluded that in some cases a symbiotic relationship may exist between cellulose-decomposing organisms and nitrogen-fixing bacteria (55, 109).

Dehérain (20) was the first to report that in the manure heap two groups of organisms are active in the anaerobic break-down of cellulose; these organisms were believed to be derived from the faecal excreta of the animals. The temperature of the heap rises only in the presence of oxygen. Hébert (36) found that about a half of the cellulose was decomposed in the manure heap within three months (49).

In the preparation of composts of horse manure for mushroom production, it is essential to reduce the cellulose concentration of the manure to a certain minimum; this can be accomplished only by proper methods of composting (122, 125); otherwise, the microorganisms of the manure developing in the beds at the expense of the cellulose may exert injurious effects upon the growth of the cultivated mushroom. When straw or other cellulose-rich waste materials are used for preparation of the composts, it is necessary not only to add sufficient moisture and to aerate the composts properly, so as to make the conditions favorable for aerobic cellulose-decomposing organisms, but also to introduce necessary amounts of available nitrogen and to some extent of phosphorus and lime. These nutrients are required for growth of the fungi, actinomycetes and bacteria which break down the cellulose and the hemicelluloses. In the preparation of "artificial composts" from

straw, the common practice has been to add about 0.7% combined nitrogen (82, 45), a quantity just sufficient to produce a good compost in a short time.

Among the special problems involved in decomposition of cellulose in straw, the process of self-heating of hay and of peat is of particular interest. It is commonly believed (66, 13) that microorganisms bring about the first stages of decomposition of the cellulose and other carbohydrates; this is followed later, after the temperature is raised sufficiently, by chemical reactions which result in actual burning. Norman (70) obtained a rise in temperature up to 49° C. as a result of the growth of pure cultures of fungi upon sterile straw; evolution of CO₂ was parallel to production of heat. Issatchenko (48) recently directed attention to the close correlation between cellulose-decomposing microorganisms and self-heating of peat.

When cellulose-rich materials are plowed into the soil, impoverishment in available nitrogen takes place, with the result that the growing crop suffers in competition with the cellulose-decomposing microorganisms (69, 114, 123). Addition of inorganic salts of nitrogen prevents this injurious effect and hastens considerably the rate of decomposition of the cellulose (15, 5), with the result that it becomes an important source of humus in soil (116).

Peat bogs and water basins—origin of coal. The rate of destruction of cellulose in peat bogs and in swamps, where standing waters prevent rapid exchange of oxygen, resulting in anaerobic systems, is considerably slower than in normal soils. The specific vegetation of the bog, the nature of its waters (presence of minerals) and climatic conditions considerably influence the rapidity and nature of decomposition. As a result of these differences various types of peat have originated—sphagnum, sedge and reed, forest and sedimentary peat. Prehistoric peat bogs gave rise to coal formations of different epochs, ranging from the older or anthracite coals to the more recent lignitic and bituminous coals. Some peats, as the lowmoor and sedimentary formations, contain very little cellulose or none; others, as the highmoor and forest peats, still contain considerable amounts. Coals, however, are virtually free from cellulose. The microorganisms concerned in destruction of cellulose under these conditions comprise largely bacteria of the anaerobic type. Formation of gases by these organisms is characteristic

of bog formation. Fungi, actinomycetes, aerobic bacteria and myxobacteria may participate in the initial stages of cellulose decomposition until the point of submergence when anaerobic conditions bring about a change in the microflora from aerobic to anaerobic.

Because of the great abundance on this planet of coal produced during different geological periods, some investigators have been inclined to believe that this material represents fossilized or transformation products of cellulose. These beliefs were substantiated by the fact that various coals contain numerous readily recognizable plant remains, some of which, especially in the younger brown coals and lignites, may show small amounts of cellulose. Evidence based upon a study of the chemical nature of coal, however, revealed that among the various plant constituents, lignins, together with certain other constituents, have contributed largely to coal formation (30). The lignins, resins and waxes are highly resistant to decomposition, especially under the anaerobic conditions which have prevailed in bogs where coal has formed. These chemical complexes were supplemented by proteins and certain carbohydrates synthesized by bacteria, with the result that a gradual enrichment has taken place in lignins, waxes and proteins, and a corresponding reduction in cellulose and hemicelluloses, finally giving rise to peat and later to coal (32). The rôle of cellulose in coal formation was thus indirect rather than direct. This is further substantiated by the fact that similar processes take place at the present time in the formation of peats (124). When, ages ago, the primitive peat bogs became covered with layers of sand, clay or silt and became subjected to pressure and increased temperature, the peat was gradually changed to coal. The nature of the latter depends upon the type of plants and their products which prevailed in the bog, as well as upon its age or period of development.

Decomposition of cellulose in aerated water basins, as in oceans and rivers, is much more rapid than in peat bogs. Aerobic bacteria primarily are concerned under these conditions, although anaerobic cellulose-decomposing bacteria have also been found; the classical anaerobic bacteria of Omeliansky were isolated first from river water (72). With the exception of certain special areas, most waters in seas and oceans contain a rather high oxygen con-

centration and, therefore, represent distinctly aerobic systems where cellulose decomposition can take place very rapidly. Issatchenko (47) and Rubentchik (89) isolated different forms of aerobic cellulose-decomposing bacteria from the curative muds of salt lakes and limans, and the abundance of such bacteria in the open sea has also been established (121)

Timber, paper pulp and textiles. In the destruction of lumber, timber, paper pulp and textiles, fungi play a predominant part, although in some cases, as in the laundry, bacteria may cause considerable damage. Lumber and its products represent cellulose-rich or chiefly cellulosic materials. Between the cutting of the tree and completion of the product, be it a house, a mass of pulp for the paper of a book or for other industrial uses, or a textile ready to wear, the cellulose is constantly subject to microbial decomposition when conditions become favorable. Lumber piled for months awaiting its turn in the factory, telephone posts buried in the ground, a mass of paper pulp exposed a little too long to water and air, a laundry shirt left a little too long in the bath, a finished book kept in too moist an atmosphere, the beams of a house or furniture within the house in a humid climate in an unprotected state, even nitrated cellulose when left unprotected—are all subject, one way or another, to microorganisms attacking the cellulose and thus injuring the product itself. The only preventive method is to expose the material as little as possible to a favorable condition of moisture and temperature for the microorganisms, to prevent their entrance, which may not always be possible, or to use disinfectants which check their development. A continuous warfare must thus be carried out against the destructive fungi and bacteria, insects, worms and a host of other organisms ready to decompose exposed and unprotected cellulose.

It is hardly necessary to list the numerous organisms concerned in these processes of destruction. Attention need be merely directed to the occurrence of microorganisms on papers and books and to the damage thus brought about through decomposition of the cellulose. These organisms belong to the lower fungi, actinomycetes and bacteria. The fungi were found (97) to be represented on papers and books by species belonging to the genera *Chaetomium*, *Myxotridium*, *Eidamella*, *Aspergillus*, *Aerostalagmus*, *Spicaria*, *Cephalothecium*, *Torula*, *Stachybotrys*, *Dematium*,

Cladosporium, *Stemphylium*, *Alternaria*, *Stysanus* and *Fusarium*. Most of these organisms are active cellulose-decomposing forms and have been isolated from other substrates. Many are pigment-forming. They bring about not only discoloration of the paper but also its perforation. The production on paper of white colonies by species of *Actinomyces* has also been reported (97). A number of other microorganisms have been demonstrated to occur on paper (113), although their rôle in the destruction of the paper has not always been established.

The organisms growing on manufactured paper (90) produce yellow, brown and black spots, while others form colorless colonies, not readily detected during the early stages of decomposition. Their action may be very slow, several months to two years elapsing before the damage can be detected by ordinary means. In some infecting agents, the extent of cellulose destruction is limited to their actual growth, while others produce enzymes which dissolve a wide zone of the cellulose surrounding the colony. The causes of infection must be suppressed in fabrication of the paper. The infection is usually introduced by insufficient sterilization of the pulp, by washing with infected water and by drying the paper with insufficiently disinfected air (23). Fungal growths upon the paper pulp can be combatted also by chlorinating the circulation waters (74).

Wood pulp may also depreciate considerably during storage. This was at first believed to be due to insect larvae, but more careful studies revealed that fungi are primarily concerned. Some attack the cellulose, some stain it, while others attack only the impurities in the pulp and may, therefore, be non-injurious. The total losses in weight of the pulp due to fungi may be as much as 27% in 6 months and 50% in 12 months (60). The air was found to be a more important source of contamination than the timber itself or the water (87). A detailed study of 46 fungi isolated from wood revealed (33a) that 38 were injurious, especially species of *Haplosporella* and *Phialophora*, whereas certain species of *Fusarium*, *Dactylium* and *Rhizopus* did less damage. Temperature, light, humidity, aeration and reaction of substrate, as well as the chemical composition of the pulp, were recognized as the most important factors influencing the injurious action of the organisms.

Various microorganisms, including both fungi and bacteria, are

also capable of causing considerable injury to cotton and other cellulose fibers. One of the most common forms of injury is that of "mildewing" which leads to discoloration of the fiber and is frequently accompanied by its decomposition (12, 96, 87*a*). The nature of the microbes attacking the cloth depends upon environmental conditions: fungi are usually most prevalent. The hyphae of these organisms are attached to the individual hairs of the fiber; they finally penetrate into the cavity of the hair, filling it completely and thereby digesting the cellulose. Nitrogenous substances in the central canal of the hair and fatty substances in the outside layers greatly favor their growth. When these are removed by bleaching, the yarns become more resistant to attack.

A number of different fungi (10) causes pigments on textiles and brings about destruction of the fiber; these fungi are either derived from the raw material or are brought in by subsequent infection. Moisture, temperature and traces of nutrient elements are the factors controlling the nature and extent of contamination. Fleming and Thaysen (29) reported that a minimum moisture of about 9% in the raw cotton is necessary for the microorganisms to develop. Schepmann (93) found that moisture is the most important factor in the destruction of cellulose in jute. Only traces of nitrogen are required for the cellulose-decomposing organisms to develop, as shown for those fungi which attack paper in books (97).

In the destruction of canvas used for tent making, both fungi and bacteria may take an active part (84), while bacteria are largely concerned in the disintegration of fishing nets (21, 107). In the retting of flax and hemp, bacteria may do considerable damage to the fibers. This includes both dissolution of the pectin which binds the fibers and destruction of the cellulose (33). According to Thaysen and Bunker (106), cottons of different origins vary in their susceptibility to attack by microorganisms. Flax retted by various processes showed marked differences in rate of disintegration, dew-retted flax being more resistant than tank-retted.

It is interesting to note that cellulose acetate fabrics were (21) more resistant to attack by microorganisms than pure cellulose fibers. However, nitrated cellulose, especially in the presence of free water, is readily subject to microbial attack. Artificial silk may also be attacked (105, 106).

INDUSTRIAL UTILIZATION OF CELLULOSE DECOMPOSITION

Industrial utilization of the activities of microorganisms capable of destroying cellulose comprises: (a) anaerobic fermentations in which certain bacteria, notably thermophilic forms, are capable of producing various organic acids, alcohols and gases; (b) production of edible mushrooms and utilization of cellulose as a source of energy for conversion of inorganic forms of nitrogen into proteins which can be used for cattle feeding (81). The first offers by far the greater possibilities. Anaerobic bacteria are able to convert cellulose into butyric, valerianic and acetic acids, ethyl and other alcohols, methane, hydrogen and carbon dioxide. Aerobic bacteria and fungi usually bring about complete destruction of cellulose, without leaving much in the form of intermediary products; hence they have little to offer for the production of industrially valuable products.

AEROBIC AND ANAEROBIC CELLULOSE-DECOMPOSING BACTERIA

Although it is now definitely established that a great variety of fungi (106), actinomycetes (61, 117, 118), protozoa and certain invertebrate animals are capable of digesting cellulose, it is the bacteria that have received and continue to receive greatest attention. Since Omeliansky's work (72) on anaerobic bacteria and Van Iterson's (110) and Hutchinson and Clayton's (44) on aerobic bacteria, numerous papers have been published dealing directly or indirectly with these two groups of organisms. The confusion brought about by many of these contributions may be emphasized by directing attention to the following facts. Although the ability of aerobic bacteria to decompose cellulose has been known since 1904, it was not until 1918 that the significance of these organisms was definitely and undeniably established. The most important organism belonging to this group has been described under more names than have most other bacteria; it has been placed among the spirochaetes (44), rod-shaped bacteria (86), mycobacteria, myxobacteria (59) and even with the actinomycetes (8).

Aerobic cellulose-decomposing bacteria can now be definitely separated into at least four distinct groups: (a) long sinuous rods, belonging to the genus *Cytophaga*; (b) short bent rods of the vibron type, under *Cellvibrio*; (c) short rods designated by Winogradsky (127) as *Celfalculula*; (d) myxobacteria and myxococci.

Cytophaga comprises a group of long, slender, flexible bacteria, 3–8 μ in length and pointed at each end. These organisms use cellulose as the exclusive source of energy, with production of slimy material and of yellow, red or orange pigments. The most characteristic morphological feature of this group of bacteria is the formation, by at least some of the most common forms, of a large round body known as a sporoid or microcyst. This was largely the cause for early confusion in identification of this organism. The bacterium was probably first isolated by van Iterson (110) and Gesher (33) who believed, however, that they had mixtures of two bacteria. Hutchinson and Clayton (44) definitely established the obligate nature of this organism for cellulose decomposition and first described its life cycle. Winogradsky (127) suggested that the name *Spirochaeta cytophaga* given to this organism by Hutchinson and Clayton be changed to *Cytophaga Hutchinsoni*; however, he found that his strain was free from microcysts; he considered these, therefore, as impurities. Krzemieniewska (58) found that the organism of Winogradsky is quite different from the one described by Hutchinson and Clayton. The latter was believed to be quite distinct from other species of *Cytophaga* in its life cycle and to resemble *Myxococcus* among the myxobacteria; the cellulose-decomposing organism was, therefore, designated as *Cytophaga myxococcoides*. Germination of the microcysts and their transformation into rods was found to be influenced by the reaction of the medium, by temperature and by oxygen tension. Issatchenko (47), who confirmed these observations of the life cycle of the organism, suggested, however, that the name given to it by Winogradsky be preserved. Castelli (14) believed that this group of organisms might best be placed in the family Heliconemaceae.

Cytophaga may thus be classified with the myxobacteria. It comprises both microcyst-forming and non-microcyst-forming species. The first group includes the organisms of van Iterson, Hutchinson and Clayton, Krzemieniewski (59), Issatchenko, Im-senecki (46) and others; the second includes the forms of Winogradsky (*Cytophaga lutea*), Dubos (24), Stapp and Bortels (100) (*Cyt. silvestris*) and others. The non-cyst-forming organisms may be considered as transition forms between true bacteria and myxobacteria. Species of *Cytophaga* are commonly found in soils and in composts and can be readily isolated by appropriate methods

(120, 9, 86). The decomposition of cellulose is influenced by the nitrogen nutrition (101). These organisms do not produce any sugars or volatile acids, but form large amounts of slime. The presence of accompanying forms considerably favors their growth and ability to decompose cellulose. This fact frequently led to hardly justified assumptions of symbiotic relationships (100).

Cellvibrio includes various vibrion-shaped bacteria. These are facultative cellulose-decomposing and are capable of growing also on other substrates. Here belong *Vibrio agar-liquefaciens* of Gray and Chalmers (34), *Bacterium* CO of Dubos (24) and *Cellvibrio vulgaris* of Stapp and Bortels (100). These organisms do not form any slime; some produce green and yellow pigments, while others do not.

Cellfalcicula comprises a variety of spindle- or sickle-shaped, motile rods with pointed ends. They are specific in respect to cellulose. The numerous rod-shaped bacteria (*Cellulomonas* types), both spore-forming and non-spore-forming, described as capable of decomposing cellulose (52, 98, 56), may for the present also be included in this genus.

In addition to the genus *Cytophaga*, other genera of myxobacteria are capable of decomposing cellulose. Here belong species of *Sorangium* (*S. cellulosum*), described by Krzemieniewski (59) and Imsenecki (46), as well as various species of *Polyangium*, *Angiococcus* (67), and others.

The anaerobic cellulose-decomposing bacteria also embrace a great variety of forms, ranging from those that thrive at room temperature to thermophilic forms, from those that live in soils, composts and sewage disposal systems to those that inhabit the digestive tract of herbivorous and omnivorous animals. Information is still inconclusive and represents several debatable questions. This is of particular importance since this group of bacteria is highly significant in the digestion of cellulose in the animal system, in industrial fermentations, in the self-heating of hay, in peat formation and in anaerobic cellulose decomposition in composts. Various claims have been made concerning isolation of these bacteria in pure cultures. Some of these isolations were successful, as shown by the work of Khouvine (54), Viljoen, Fred and Peterson (115), Woodman and Stewart (129), Clausen (16) and Snieszko (99). However, Coolhaas (18) concluded in 1928 that anaerobic cellu-

lose decomposition is carried out in nature by the combined action of two bacteria, an anaerobic form which decomposes the cellulose, giving rise to intermediary compounds, and an aerobic organism which attacks the latter by a fermentation process. This assumption was in line with the earlier ideas of Kellermann and McBeth (51), as opposed to those of Omeliansky (72) and Pringsheim (79) who believed that the anaerobic bacteria were specific in bringing about the decomposition of cellulose.

Pochon (75) divided the anaerobic cellulose-decomposing bacteria into two groups on the basis of the products formed, namely, the aceto-butyric and the formo-acetic. The first group, represented by *B. cellulosa dissolvens*, occurs in human digestive systems, in rabbits and in insect larvae; these organisms are strictly anaerobic, produce a yellow pigment, require a growth-promoting substance and can not attack any other carbohydrate than cellulose. The organisms belonging to the second group are represented by *Plectridium cellulolyticum*; they occur in the digestive tract of ruminants, are facultatively anaerobic, decompose cellulose only under anaerobic conditions, attack sugars and do not produce a yellow pigment; they also require a growth-promoting substance. There are many gradations between these two groups, with various degrees of adaptation to saprophytic existence.

CELLULOSE-DECOMPOSING FUNGI AND ACTINOMYCETES

The important rôle of fungi in decomposition of cellulose in nature was become definitely established. The methods of isolation of these organisms presented comparatively little difficulty once their capacity to bring about the destruction of cellulose was recognized. In acid soils this was found (28) to be carried out largely by fungi. These organisms also play an important rôle in cellulose destruction in composts and in wood, both in the growing and in the felled tree, as well as in various forms of lumber. Their rôle in cellulose digestion by termites has also been indicated (43). Cellulose-decomposing fungi belong to a great variety of species and genera, largely among the Hyphomycetes, Ascomycetes and Basidiomycetes (106). The breakdown of cellulose in wood by fungi results in the formation of characteristic enzymatic cavities (3). Use of polarized light for direct microscopic observation of the destruction of cellulose by fungi has been suggested (4).

Numerous actinomycetes are also capable of breaking down cellulose under various conditions (118, 106).

CHEMISTRY OF CELLULOSE DECOMPOSITION

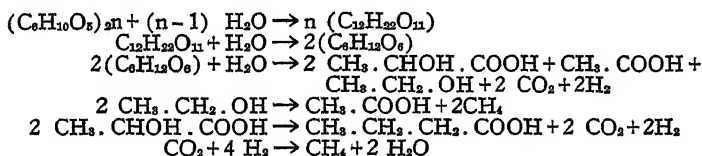
Various attempts have been made to demonstrate formation by microorganisms of enzymes concerned in hydrolysis of cellulose. Only very few of these may lay claim to success. De Bary (19), in 1886, was the first to make a careful study of dissolution of cell walls of plants by fungi; formation by these organisms of a cellulose-dissolving enzyme, later designated as cellulase, was thereby indicated. Von Euler (26) obtained from the wood-destroying fungus *Merulius lacrymans* an enzyme preparation which acted not upon cellulose itself but only upon its hydrolytic products (63). Pringsheim (79) and more recently Woodman (128, 129) claim to have found that cellulose-decomposing bacteria produce two groups of enzymes, one a cellulase which hydrolyzes cellulose to the disaccharide cellobiose, and the other a cellobiase which converts the cellobiose to glucose. The method of demonstrating the action of these enzymes consisted in allowing the growth of the bacterium to accumulate, then arresting active growth by addition of a disinfectant, such as iodoform dissolved in acetone or toluol; this was also brought about by raising the temperature to a point (70° C.) slightly above the maximum for bacterial growth. By allowing the sugar to dialyze out, as soon as formed, through a membrane, or by making the reaction of the medium alkaline (35), thus allowing sugar to accumulate, the activity of the enzyme was also demonstrated.

Simola (98) found that two aerobic cellulose-decomposing bacteria, *Cellulobacillus myxogenes* and *C. mucosus*, produced cellulase and cellobiase; the enzymes remained active, even when growth of the bacteria was stopped by addition of toluol. A cell-free enzyme extract was obtained which produced reducing sugar from cellulose. The optimum temperature for the enzymes was 37° C. and the optimum pH 6.0 to 7.0. Vartiomaara (112) found that sugar will accumulate in the decomposition of cellulose by fungi when conditions are unfavorable for growth. The question always remains whether this sugar may not possibly be derived by autolysis of the fungus mycelium.

The occurrence of cellulolytic enzymes in lower animals and in

germinating seeds has also been demonstrated. Karrer (51) has shown that cellulase is present in the digestive system of the edible snail (*Helix pomata*). The action of this enzyme was studied primarily upon lichenin, a carbohydrate which is looked upon as a "reserve cellulose." It has also been claimed (10) that the anterior portion of the shipworm, *Bankia setacea*, contains cellulase. The ability of termites to digest cellulose has been ascribed to the protozoa living in their intestinal tract (17). These termites, as well as the wood-feeding roach *Cryptocercus punctulatus*, were found (108) to produce cellulase; this enzyme has also been obtained from one of the intestinal flagellates, *Trichomonas termo-pridis*, which was grown on a cellulose medium. The enzyme could be adsorbed on aluminum hydroxide and eluted with 3% K_2HPO_4 . Various cultures of cellulose-decomposing bacteria have been isolated from the gut of termites; these bacteria were found to belong largely to the anaerobic group (43).

The glucose produced in the hydrolysis of cellulose by microorganisms is further broken down, in the process of microbial nutrition, to simpler compounds. In the case of anaerobic bacteria, these are found to be the characteristic products of fermentation reactions, as elucidated by Langwell and Lymn (62) and Neuberg and Cohn (71):



Although these reactions are largely hypothetical, they serve, nevertheless, to explain the processes involved in the formation of the various acids, alcohols and gases in anaerobic decomposition of cellulose.

Popoff (76) was the first to analyze the gases given off in the decomposition of cellulose under anaerobic conditions, and found them to be rich in methane under a slight admixture of hydrogen. Hoppe-Seyler (39) used filter paper as a source of cellulose, and obtained hydrogen and methane among the gaseous products of fermentation. The formation of acids and alcohols was also observed in the early studies. Van Senus (111) suggested that

methane and ethyl alcohol are not the primary fermentation products, but are due to the reduction of acetic acid by hydrogen, the last two being the primary products.

Omeliansky (72) demonstrated a quantitative transformation of cellulose, under anaerobic conditions, into fatty acids (acetic, butyric and valerianic) and gases (CO_2 and H_2 or CH_4). Out of 3 grams of cellulose decomposed, Pringsheim (80) obtained 0.213 gm. formic acid and 1.15 gm. acetic acid, as well as a small amount of lactic acid; the gases consisted of CO_2 (22–49%) and hydrogen. In Khouvine's experiments (54), ethyl alcohol, acetic acid, butyric acid, traces of lactic acid, CO_2 and H_2 were found. Out of 42 grams of cellulose decomposed, Viljoen, Fred and Peterson (114) reported the formation of 21.6 gm. acetic acid, 10.3 gm. alcohol and 11.0 gm. CO_2 , in addition to hydrogen. Glucose was also demonstrated among the products of cellulose decomposition, frequently in considerable amounts (95,103).

Less is known of the mechanism of cellulose decomposition by aerobic bacteria and fungi, since only seldom have intermediary substances been isolated in these investigations. Kalnins (50) reported, however, that as much as 30% of the cellulose added to a culture of *Bacterium protozoides* was converted to a compound reducing Fehling's solution; limitation of the oxygen supply increased the accumulation of this compound. Among the final products of aerobic decomposition, the formation of slimy material, pigments and synthesized microbial cell substance has usually been observed (127). Winogradsky suggested that the slimy material is of the nature of oxy-cellulose, which he considered as evidence that it is a direct oxidation product of the cellulose. Marcusson (64) utilized this suggestion in formulating an hypothesis concerning the rôle of cellulose in coal formation. He believed that cellulose is first oxidized to "oxy-cellulose," which gives rise to "humal acid" which, in turn, condenses to form "humic acid" and coal. This purely hypothetical conception was not based upon facts. The existence of oxy-cellulose itself has never been established, since the available evidence seems to indicate that the slimy material produced in bacterial cultures is not an oxidation product of cellulose but a synthetic complex formed by the microbial cell. This material has been found, in various bacteria, to contain uronic acids, a fact pointing to the synthetic nature of the material. The same is true of the pigments produced by the aerobic bacteria.

The major product of aerobic cellulose decomposition is, in addition to CO_2 , synthesized bacterial cell substance. The aerobic bacteria and fungi bring about rapid and complete oxidation of the cellulose to CO_2 and water, with the possible formation of sugars or dextrin-like substances as intermediary products, thus making a large amount of energy available to the organisms. This allows extensive synthesis of microbial cell substance, which can best be measured by the amount of inorganic nitrogen converted into microbial protein. A cellulose-nitrogen ratio of 30:1 has been demonstrated for pure cultures of fungi and bacteria, and a ratio of 50:1 for mixed populations (38). Assuming that the organisms contain 5 to 10% nitrogen, the ratio between cellulose decomposed to synthesized cell material becomes 30:10 to 30:20. Since the carbon content of microbial cell substance (50% carbon) is higher than that of cellulose (40% carbon), it is possible to demonstrate that as much as 40 to 70% of the carbon of the cellulose decomposed may be converted into microbial cells. This phenomenon is of considerable significance in any concept developed in regard to the origin and nature of humus.

ENVIRONMENTAL CONDITIONS AND CELLULOSE DECOMPOSITION

Among environmental factors which influence the nature of the microorganisms active in the destruction of cellulose under a certain set of conditions, moisture, reaction, aeration and temperature, as well as the supply of available nitrogen and other nutrient elements, are particularly important.

A high moisture content (80–95% of substrate) was found to favor anaerobic bacteria and to be injurious to the development of fungi and most actinomycetes. Medium moisture (60–75%) is favorable to fungi and to aerobic cellulose-decomposing bacteria. Very low moisture (8% or less) completely stops the activities of most organisms, although some may still be able to make a certain amount of growth even under these conditions.

The reaction of the medium also has a marked influence upon the nature of the flora responsible for the decomposition of cellulose. Although Hutchinson and Clayton (44) claimed that the aerobic bacterium belonging to the *Cytophaga* group is able to grow at a wide range of reactions, Thaysen and Bunker (105) could not obtain any growth at a pH less than 6.1 and greater than 9.1.

Dubos (25) could not demonstrate the presence of this organism in soils more acid than pH 5.4–5.8, but found it very abundantly in only slightly acid, as well as in neutral and in alkaline soil; he has further shown that, whereas *Cytophaga* and other cellulose-decomposing bacteria develop at pH 6.0–9.0, *Actinomyces* sp. grow at pH 5.5–9.5; fungi grow at a wide reaction range, namely pH 3.0–9.5. Some cellulose-decomposing fungi, like *Trichoderma*, are able to grow even at as high an acidity as pH 2.1–2.5. A slightly alkaline reaction (pH 7.5) favors, therefore, the growth of bacteria, whereas an acid reaction is injurious to bacteria and is favorable to fungi. Addition to the soil of acid-reacting fertilizers which result in a low pH favors development of fungi concerned in cellulose decomposition; addition of alkaline fertilizers, especially lime, considerably reduces the numbers of fungi and favors bacterial decomposition of cellulose (28).

The common aerobic cellulose-decomposing bacteria have their temperature optimum at 20°–28° C. (25); the anaerobic organisms grow best at 37° C. (54); the thermophilic fungi, bacteria and actinomycetes grow at 50°–65° C. (125). Different temperatures thus favor the development of different groups of organisms, thereby modifying the nature and extent of cellulose decomposition.

Oxygen supply also influences both the nature of the cellulose-decomposing flora and the rate of decomposition. When horse manure has to be stored for some time, since it may not as yet be needed for preparation of composts for mushroom production, it has to be kept well compacted, thereby resulting in only very little decomposition. However, 5–6 weeks before the compost is needed for the mushroom house, it is thoroughly aerated; this leads to active decomposition which is accompanied by rapid destruction of the cellulose and by a rise in temperature.

The need of available nitrogen and mineral nutrients for the microorganisms decomposing cellulose has a number of important aspects. The presence of these compounds hastens cellulose destruction, with the result that inorganic forms of nitrogen are transformed into complex organic compounds, giving rise to humus. The formation of a good compost and the conservation of nitrogen in stable manures are based upon this phenomenon. When cellulose is destroyed in fabrics and in timbers, the presence of small amounts of nutrients is also favorable to the process; these ele-

ments must, therefore, be eliminated in order to conserve the cellulose. Unfortunately, many wood-destroying fungi can thrive well even with very little nitrogen, whereas exposure of wood, timber and canvas tents to rain renders some of the combined forms of nitrogen in the atmosphere available for these organisms.

Among the factors which prevent cellulose decomposition, lignin occupies an important place. Many organisms are able to attack wood only very slowly because of the high lignin content. Removal of lignin renders the cellulose more digestable to microorganisms and higher animals (77). Rege (85) spoke of lignin as an "inhibitory factor" in cellulose decomposition. Some higher fungi, however, are capable of attacking cellulose even in the presence of considerable lignin.

PROBLEMS IN CELLULOSE DECOMPOSITION STILL REMAINING UNSOLVED

Although the accumulated knowledge of microorganisms responsible for destruction of cellulose in nature is already very extensive, a number of problems still remain unsolved; some of these are of great economic importance:

1. A better knowledge of the formation of specific enzymes responsible for degradation of cellulose to simpler compounds; methods of obtaining from microorganisms preparations of cellulolytic enzymes.

2. Better understanding of the chemical processes involved in decomposition of cellulose under anaerobic *vs.* aerobic conditions.

3. The nature of anaerobic cellulose-decomposing bacteria, and their relation to aerobic organisms and to possible symbionts.

4. The rôle of bacteria in the digestion of cellulose by herbivorous and omnivorous animals, by insects and by wood-destroying larvae and worms.

5. The specific rôle played by cellulose-decomposing fungi in the causation of plant diseases.

6. The utilization of cellulose-decomposition organisms for the production of industrially important substances, such as alcohols, acids and gases.

7. The ability of nitrogen-fixing bacteria to utilize the products of cellulose decomposition as sources of energy for nitrogen fixation.

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THE DEVELOPMENT OF SPHAGNUM BOGS IN NORTH AMERICA¹

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The bogs discussed in this paper are those that have a fairly continuous growth of sphagnum moss at the surface which has formed a layer of peat of appreciable thickness and which has exercised a selective influence on the community of plants growing in it. Bogs of this kind are common across the continent from the Atlantic to the Pacific in northern United States, including Alaska, and in southern Canada. They are, in general, more abundant and extensive along the coasts than in the interior. The separation of these bogs from the vast muskegs and tundras to the north of them is not definite (Rigg 1937, 1940). Toward the south they become less and less abundant and are found only at higher elevations or where local topography and climatic conditions are especially favorable. In the United States they are common on the Atlantic coast in the New England states (Bastin and Davis 1909; Nichols 1915, 1919; Dachnowski 1929) and New Jersey (Rigg 1940) and on the Pacific coast in the western portions of Washington and Oregon (Rigg 1925, 1940; Rigg and Richardson 1934, 1938; Dachnowski 1930). In the interior they are found in the States lying between the groups just mentioned but are less abundant in the drier states (*e.g.*, Montana, Wyoming and Colorado) than in the states having a higher rainfall (*e.g.*, Minnesota, Michigan, Ohio and Pennsylvania). They are also abundant and extensive in Alaska (Rigg 1914, 1937, 1940). In Canada they are numerous and large on the Pacific coast in British Columbia (Rigg and Richardson 1938) and on the Atlantic coast in Nova Scotia, New Brunswick and Quebec (Gannong 1891, 1897; Auer 1926, 1927, 1927*a*, 1930; Anrep 1915; Nichols 1918). In the interior of Canada they are common wherever conditions are favorable at about the same latitudes as on the coasts or a little farther north (Lewis and Dowding 1926; Lewis, Dowding, and Moss 1928). The farthest south bog known on the Pacific coast is at Fort Bragg in northern California (Rigg 1933).

¹ This paper deals with the course of development of the bogs themselves and only incidentally with pollen analysis as an evidence of the development of vegetation in the regions surrounding the bogs.

They occur in undrained or poorly drained places and their best development is favored by a relatively cool and moist climate with the absence of great extremes of heat and cold. Unless some local factors either limit or favor their development, large and numerous sphagnum bogs are more likely to be found in oceanic climates than in continental ones. *Sphagnum* requires cool, moist conditions for its growth, and these conditions may characterize regions of large area or may occur locally. It occurs even in southern United States where local conditions are favorable. Five species are reported in one county in Florida (Murrill 1938). Two of these (*S. palustre* and *S. recurvum*) are abundant.

In considering the occurrence of sphagnum bogs in the United States the enormous peat areas of Minnesota deserve special attention. That state has approximately 7,000,000 acres of peat lands, over 5,000,000 of which is covered with peat at least 5 feet thick and some of it with peat 30 feet or more in depth (Alway 1920). Most of this is in the northern portion of the state. A small portion of it is occupied by wild grasses and sedges, but most of it is covered with dwarf trees. Soper (1919) speaks of Minnesota sphagnum-heath bogs which are "composed essentially of sphagnum moss from top to bottom" and "were built up on flat undrained areas" and "occur chiefly in the north especially within the area formerly occupied by glacial Lake Agassiz." He speaks also of sphagnum-pond bogs "which have become covered with sphagnum peat after the lake was filled . . . with deposits consisting essentially of pondweeds (*Potamogeton*) and other aquatic plants such as pond-lilies, lake bulrushes, amphibious sedges, etc." This indicates that *Sphagnum* has flourished extensively in northern Minnesota and has played an important role in bog development. Field work by the writer (Rigg 1940) on Minnesota bogs in the vicinity of Lake Itasca, however, indicates that *Sphagnum* has played a smaller part in the development of these bogs than it has in bogs of the Atlantic coast and the Pacific coast.

SPHAGNUM AND THE CONDITIONS THAT IT CREATES

Sphagnum as a living plant has certain distinctive characters of structure and growth habits. Dead sphagnum also has pronounced physical and chemical properties. These characters and properties play their part in enabling this plant to develop a habitat in which

certain plants flourish while others are excluded. There are many species in the genus (Trelease 1910; Frye 1918; Paul 1924) but they all have several points in common in which they differ from all other plants. In some points this is a difference of degree only (quantitative), while in others it is clearly a qualitative difference. The long, slender leafy stems grow vigorously at the tip and die at the basal portion without immediate disintegration. The propagation of the plants is largely vegetative and the occurrence of the sporophyte generation is, so far as the writer has seen, relatively rare, though it is occasionally found in abundance in small areas. Woesler (1934) found that vegetative reproduction of *Sphagnum cymbifolium* by means of filaments, plates of cells, and buds occurs naturally from growing tips of stems and from fragments of young leaves. He found experimentally that vegetative reproduction from short branches and even from older leaves occurred freely under high atmospheric humidity and moderate light and that it was increased by wounding. He cites the papers of several workers who had earlier observed vegetative reproduction in other species of *Sphagnum* and had developed methods of showing it experimentally. It does not seem probable that rhizoids play any large part in anchorage, but rather that the plants hold their position by their relation to one another. Since the cellular structure of the plants is such that water is readily taken up by any portion of the plant it seems improbable also that the rhizoids play a major role in the intake of water.

Both stems and leaves of *Sphagnum* contain large cells whose active portion dies by the time the cells are mature, leaving only the wall which is characterized by small pores and is supported by ring-like thickenings on the inside. These are known as hyaline cells, and their bulk is much greater than that of the ordinary living green cells. These hyaline cells take up and hold large quantities of water. Almost any species of *Sphagnum* will take in and hold ten times its own weight of water and the more robust and leafy species may hold twice that much. Shibata and Watanabe (1932, 1934) have determined cellulose (60.49%), lignin (14.51%), pentosans (14.78%), and ash (3.99%) in sphagnum and have found galactan, mannan and crude protein. Goheen (1939) has demonstrated the presence of cellulose and pectin in sphagnum.

Sphagnum causes water with which it remains in contact to

have an acid reaction. This is true not only of living sphagnum but also of its dead remains. Bauman and Gully (1910) expressed the idea that hydrogen ions are set free by exchanges of ions between the cells of *Sphagnum* and the bathing solution. Baas-Becking and Nicolai (1934) approve this theory and find by experiment that *Sphagnum cymbifolium* does not excrete acid when grown in distilled water. They consider the exchange theory to be the only one which may account for the observed facts in regard to the acidity of the waters of sphagnum bogs. They state "Bauman and Gully consider the cell wall to be a colloid which, when placed in a salt solution, adsorbs the kations exclusively and sets free the acid". The following suggestion in regard to the reaction involved has been made to the writer by Dr. H. E. Wirth. "If positive ions (*e.g.*, Na⁺) are adsorbed on a surface they will attract negative ions (*e.g.*, OH⁻ of water) and form a "double layer" on the surface. The removal of the OH⁻ ions from activity in the water leaves an excess of H⁺ ions." Skene (1915) obtained a marked decrease of the pH by growing *Sphagnum* in solutions of sodium chloride. Alway and Nygard (1927) have distinguished between lime-deficiency and acidity in peat soils.

Williams and Thompson (1936) found that dead sphagnum, even after it had been washed in 95% alcohol in a Soxhlet extractor, then dried, shredded, and passed through a 20 mesh sieve increased the hydrogen ion concentration of salt solutions to which it was added. The greater the concentration of the salt solution the greater was the increase of the hydrogen ion concentration upon the addition of the sphagnum, and with a given salt solution, the hydrogen ion concentration increased with the quantity of sphagnum added. They conclude that "It seems probable that the adsorption of metallic and hydroxyl ions explains, at least in part, the acidity of the waters of sphagnum bogs."

It is well known that the waters of sphagnum bogs are acid. Baas-Becking and Nicolai (1934) found that the pH of water of a bog in Holland was 3.7 to 4.1, the maximum being in the open water of a bog just after a rain storm. Thompson, Lorah, and Rigg (1927) found the hydrogen ion concentration of the waters of various sphagnum bogs near Seattle, Washington, expressed as pH to vary from 3.83 to 4.93. They concluded that "While the acidity of bog waters of the Puget Sound region may be due to car-

bonic acid, it is not the dominating cause of the acidity" and also that "The amount of organic matter . . . seems to be the controlling factor of acidity" and that "There appears to be a rather indefinite relationship between color intensity and acidity." It was early suggested that the acidity of the waters of sphagnum bogs was due to carbon dioxide, but since the acidities found are greater than could be produced by the reactions of carbon dioxide with water, and since only a portion of the acidity can be removed by boiling, it is evident that carbon dioxide is only one factor in the acidity of bog water and not usually the dominant one. The conclusions of Baas-Becking and Nicolai (1934) for a bog in Holland agree with those of Thompson, Lorah, and Rigg for bogs of western United States. The former say, "The conclusion seems, therefore, warranted that in the insufficiently buffered milieu the carbon dioxide will cause a certain decrease of pH, but in no case the low pH should be attributed exclusively (or even for an important part) to the presence of carbon dioxide." Since sphagnum causes acidity rather than requiring acid conditions for its growth, it is not surprising that where sphagnum bogs border large lakes or even float upon them, the bog should be acid, while the lake is alkaline.

Bog water has a distinct color, varying from yellow to brown. It is often spoken of as being "coffee colored." It has a very low osmotic equivalent. Several workers have determined the osmotic properties of bog water. Livingston (1904) says that "bog waters do not have an appreciably higher concentration of dissolved substances than do the streams and lakes of the same region," and Fitting (1911) and Rigg, Trumbull, and Lincoln (1916) agree with this. All the determinations of the lowering of the freezing point show osmotic pressures of far less than one atmosphere.

The colloidal properties of bog water have been studied by Rigg and Thompson (1919). They found that a dark colored precipitate was obtained from bog water by treatment with ammonium sulphate, and also with other electrolytes, leaving the bog water colorless. Baas-Becking and Nicolai (1934) say "The presence of small amounts of organic acids in the water of bogs seems a well established fact." These workers have made a very thorough study of the physiological and ecological conditions in a sphagnum bog, including the presence and activities of bacteria in the water and substratum, and have given a schematic representation of the life cycle in a sphagnum bog.

It must not be assumed that all the properties of the water in the sphagnum layer are due wholly to the presence of sphagnum and its products, because the activities of the roots of other plants growing there must be considered, as must also the activities of micro-organisms. Undoubtedly, however, most of the properties of the water in the sphagnum layer can be traced directly or indirectly to the sphagnum. Summarizing the data given above we may say that the water on which the roots of plants growing in sphagnum bogs must depend is a colored, colloidal solution of rather high acidity, low osmotic pressure, and low mineral content, and that it has a high content of carbon dioxide and a low content of oxygen.

The idea that bog water is toxic has been expressed by Livingston (1904, 1905), Dachnowski (1908) and the writer (Rigg 1913, 1916), and experimental data have been obtained that tend to support this view. The writer wishes to make the criticism of his own very early work on this subject that the water in his cultures was not changed; hence the oxygen content may have become abnormally low and the carbon dioxide content abnormally high even for bog water. It is possible too that the toxicity may be expressed in terms of some of the physico-chemical properties of bog water stated above. Alway (1920) finds that some peat soils of Minnesota have a toxic substratum due to the presence of ferrous sulphate and sulphuric acid, but he states that these two substances do not ordinarily occur in wild, undrained bogs. He says they appear soon after drainage due to the oxidation by the air of the original iron sulphide (iron pyrites).

BOG PLANTS²

Certain species of plants occur so commonly in sphagnum bogs that they are known as bog plants. Among the well known bog herbs are the round-leaved sundew (*Drosera rotundifolia*), the long-leaved sundew (*D. anglica*), several species of cotton grass (*Eriophorum Chammissonis*, *E. gracile*, *E. callitrix*, *E. angustifolium* and *E. tenellum*), the white-beaked rush (*Rhynchospora alba*), two pitcher plants (*Sarracenia purpurea* and *Darlingtonia*

² The botanical names of Spermatophytes used in this paper are according to Gray (1908) for all names occurring in that work. All others are according to Piper and Beattie (1915) except *Lysichitum americanum* which is according to Hulten and St. John (1921).

californica), the cloud berry or baked-apple berry (*Rubus chamaemorus*), two species of skunk cabbage (*Symplocarpus foetidus* and *Lysichitum americanum*), the creeping snowberry (*Chiogenes hispidula*), at least two gentians (*Gentiana Douglasiana* and *G. sceptrum*), the buckbean (*Menyanthes trifoliata*), the marsh cinquefoil (*Potentilla palustris*), several sedges (*Carex trisperma*, *C. tenuiflora*, *C. tenella*, *C. diandra*, *C. leptalea*, *C. limosa*, *C. livida*, *C. oligosperma* and other species), a number of mosses (*Sphagnum*, *Hypnum*, *Caliergon*, *Hylocomium*, *Polytrichum*, and other genera) and some lichens (*Cetraria islandica* and others).

Some shrubs, including woody vines, characteristic of sphagnum bogs are two species of Labrador tea (*Ledum groenlandicum* and *L. columbianum*), pale laurel (*Kalmia polifolia*), sheep laurel (*K. angustifolia*), leather leaf (*Chamaedaphne calyculata*), bog rosemary (*Andromeda glaucophylla*), large cranberry (*Vaccinium macrocarpon*), small cranberry (*V. Oxycoccus*), sweet gale (*Myrica Gale*) and its congener (*M. californicum*), and two birches (*Betula glandulosa* and *B. pumila* var. *glandulifera*).

Among the trees that commonly invade bogs are white cedar (*Chamaecyparis thyoides*), black tamarack (*Larix laricina*), Sitka spruce (*Picea sitchensis*), bog spruce (*Picea mariana*), western hemlock (*Tsuga heterophylla*), three pines (*Pinus rigida*, *P. contorta*, and *P. monticola*), the red maple (*Acer rubrum*), and at least one birch (*Betula occidentalis*).

Some plants which occur in sphagnum bogs are survivals from the swamp or even from the lake which preceded the bog, and are found in only the earlier stages of bog development. In bogs of the Pacific coast *Darlingtonia* (Rigg 1925) and *Lysichitum* (Tureson 1916) are obviously swamp plants which endure the sphagnum for a time but are eventually killed out by it. *Nymphaea* which flourished in a lake often survives in the earlier sphagnum stages.

Some plants which are common even in mature sphagnum bogs flourish also in wet places in the same region. A good example of this in the Puget Sound region is *Ledum groenlandicum* which often grows tall and forms dense thickets in swamps where other bog plants are absent.

Some plants which grow in great abundance in northern regions are found in temperate regions only on mountains and in sphagnum bogs. The crow berry (*Empetrum nigrum*) is common near sea

level in Alaska and is found in the State of Washington only on mountains and in sphagnum bogs, and occurs in occasional patches in bogs as far south as the Coos Bay region in Oregon. It is generally believed that bog societies, since they are composed largely of boreal species, are relicts of former climatic conditions. The opinion of Transeau (1903) agrees with that of the writer on this point. Some bog shrubs show xerophytic structure in their leaves. *Ledum groenlandicum* is an extreme example. Its leaves are in-rolled at the margin and have a thick dense covering of woolly hair on the ventral surface. They have no stomates on the dorsal surface and those on the ventral surface are sunken beneath the level of the epidermis.

THE KINDS OF PEAT FOUND IN SPHAGNUM BOGS

Bogs are composed of stratified peat, and peat may be characterized (Bulow 1929) as a mass of incompletely decomposed plant parts, dark colored, rich in carbon and more or less acid. Though peat is composed largely of organic matter it carries a variable amount of mineral matter partly taken up by the plants while growing, but largely derived from the dust blown or the mud washed into the bog or lake as the peat was being formed (Alway 1920). A considerable number of different kinds of peat form layers in bogs. A comprehensive list of these with a statement of their characteristic properties and the plants from which they originate has been given by Bulow (1929) in his world-wide discussion of bogs. The origin and character of the various kinds of peat found in bogs of southeastern Canada has been given by Auer (1930). The peat deposits of Ohio have been treated by Dachnowski (1912), and the origin and characteristics of the various kinds of peat found in bogs of the United States have been comprehensively discussed by Dachnowski-Stokes (1933). The peat and muck deposits of Vermont have been described by Hills and Hollister (1912). A statement of the characteristics of peat in comparison with mineral soils, accompanied by tables giving data for Minnesota, has been made by Alway (1920). Peat strata in bogs (mainly of the Pacific coast) have been described by Rigg (1925, 1940) and Rigg and Richardson (1934, 1938). This is by no means a complete list of papers dealing with the kinds of peat found in bogs of North America, but the extensive literature lists in the papers cited include many of them.

Sphagnum moss frequently forms pure strata in which no other plants are present at all. In some strata the moss shows very little disintegration, entire leafy stems being found intact. In other cases the stems are in fragments and many of the leaves are separated from them. Strata of sphagnum peat in these two states are frequently found in contact, with a sharp line of demarcation between them. Other strata show more complete disintegration of the moss. Sphagnum peat is commonly characterized on the well known von Post scale in which number one is peat showing no disintegration and from which, when squeezed in the hand, nothing but water comes out between the fingers, while number ten is material that is so completely disintegrated that no sphagnum remains are identifiable with the unaided eye and when squeezed in the hand all the material comes out between the fingers. The numbers two to nine represent stages between these two extremes. Sphagnum peat, however, is not always pure and it may contain other mosses as well as the remains of roots, stems and leaves of other plants.

Sedge peat consists mainly of the remains of leaves, stems, and roots of sedges (*Carex*), mostly macroscopic, but in some cases disintegrated into very small particles. It is not commonly pure sedge, but contains in varying amounts the remains of various swamp plants as well as particles of disintegrated wood, grasses, *Sphagnum* and other mosses, and also intact coniferous needles and heath leaves. The microscopic remains are less abundant and consist largely of diatoms, pollen, finely divided remains of plant tissues, sponge spicules and the harder portions of small animals. Chemical analysis of four samples of sedge peat from a bog near Seattle gave an average moisture content of 87.3%. On a dry weight basis they showed the following averages—nitrogen 1.88%; calcium as CaO 0.13%, phosphorus as P_2O_5 0.16%, potassium as K_2O all less than 0.2%.

Wood peat consists of the remains of trees and shrubs. Some of it is fairly compact, but much of it is so watery that it is difficult to secure a sample with a peat borer. Logs and stumps are often found. Reed peat consists of the remains of reeds, mainly *Phragmites*. It may form a separate layer or it may be much mixed with sedges. It is frequently found mixed with tules (*Scirpus*) and is then called tule-reed peat. Fairly distinct layers of peat are formed in some bogs from the macroscopic remains of other plants. Peats

originating from *Potamogeton*, *Nymphaea*, *Equisetum*, *Menyanthes*, *Rhynchospora*, *Eriophorum* and *Scheuchzeria* have been found in North American bogs, and still other kinds are reported by workers in Europe and Asia.

Sedimentary peat consists of finely divided particles of material deposited from water. It is distinguished from lake mud by being usually lighter in color and containing a higher percentage of organic matter. The material composing it has originated largely from organisms that lived in the pond or lake which preceded the bog, though it often contains some lake mud which has been brought in by drainage from the surrounding slopes and undoubtedly also some material blown in by winds. The amount of macroscopic material in it is small, consisting mostly of needles of conifers, fragments of wood, and remains of sphagnum, other mosses, and sedges. Lake mud consists largely of material that has been washed in from the surrounding slopes though much of it has received some organic material from the water. A few samples from the Puget Sound region showed an average of 82.4% of moisture, and the dried material lost 34% of its weight on ignition. Occasional fragments of wood, sedge, and sphagnum have been found in it. Some samples show diatoms, algal filaments and pollen grains. A layer of jelly-like colloidal material of yellowish color is found in some bogs. In Cottage Lake bog near Seattle a layer of this material over four feet thick extends throughout the bog. It seems quite possible that it has originated from algae.

Muck is common in sphagnum bogs, especially at the margin. In speaking of the term muck, Alway (1920) says that ". . . technically it is generally applied only to those peats in which there is a high proportion of mineral matter or the plant residues are so thoroughly decomposed that little or none of their original structure is recognizable," and in his bulletin he confines the term to peat soils containing more than 50% ash. It is apparent that there is some overlapping of properties in lake mud, muck and sedimentary peat, and in field work the line dividing any two of them is not always sharp. They differ sufficiently, however, so that the use of the three terms is advantageous in describing the course of bog development.

A layer of volcanic ash is present in many bogs. Rigg and Richardson (1934, 1938) have discussed this and have shown it

in profiles of bogs of the Puget Sound region. The layer is usually only an inch or two in thickness in bogs of the Pacific coast, though it is occasionally as much as six inches, and in a few bogs two layers separated by a thick layer of peat are found. The layer of ash is usually in sedimentary or sedge peat, occasionally in lake mud. Auer (1933) shows three separate layers in two bogs of Terra del Fuego. Hansen (1940) has found that crystals of volcanic ash are sometimes recognizable in sphagnum peat even where no definite layer of ash is found. In a bog in British Columbia he found the crystals most abundant at the 2.5 meter level, though they extended from 2.25 to 2.75. He suggests that the ash fell on a sphagnum moss cover and was washed down by rain.

The most common material on which sphagnum bogs rest is blue clay. A few bogs in rocky regions rest on rock, and in sand dune areas where the sand is full of water some of them rest directly on the sand.

RAISED BOGS AND FLAT BOGS

Two kinds of sphagnum bogs, flat and raised, are commonly recognized. In a flat bog there is very little difference in elevation between the center and margin, sometimes none at all. This statement applies only to the area occupied by the sphagnum, and there is commonly a "marginal ditch" or "mote" around the sphagnum area separating it from the neighboring higher land of ordinary soil. A raised bog is distinctly higher at the center than at the margin. A difference of as much as 15 feet is sometimes found. The bog is thus either dome-shaped or has the form of a ridge. The distinction between these two kinds of bogs is not a sharp one since many of those that look flat are really slightly higher at the center than at the margin, but still the classification is a convenient one. Some of the phenomena involved in the development of bogs which are distinctly dome- or ridge-like are different from the phenomena occurring in those whose surface, even at maturity, is relatively flat, and this furnishes an additional reason for separating raised bogs from flat bogs in giving an account of their character, origin, and development.

In Europe these two kinds of bogs have been distinguished by many workers during comparatively recent years on the basis of the character of plant nutrition in them. The statement (translated freely) by Bulow (1929, p. 1) puts this clearly and briefly:

"The usual division of moors (bogs) into raised and flat moors is in this paper used in the wider sense that flat bogs include eutrophic formations under the surface of the ground water while raised bogs include oligotrophic deposits above the surface of the ground water and only in exceptional cases under it. This does away with the two typical kinds of bogs, 'normal' flat bogs and 'true' raised bogs. The idea of intermediate bogs is not used since this group can be distinguished only from the botanical standpoint and not from the standpoint of geologic origin." In support of the idea that the conditions for plant nutrition are much more favorable in flat bogs than in raised bogs, Bulow (p. 42) gives data on the comparative mineral content of the water in the two types and also on the comparative amount of organic matter present. The amount of mineral matter in parts per 100,000 in the two is stated to be: flat bogs 16.5 and raised bogs 3.0. The following analytical data on the minerals present (p.p.m.) in the two types are given, the first number in each case being for flat bogs and the second for raised bogs; K_2O 2.285-0.595, Na_2O 0.739-0.473, CaO 6.261-1.170, MgO 0.651-0.208, MnO 0.350-trace, Fe_2O_3 4.008-0.903, P_2O_5 0.645-0.095, SO_3 0.526-0.098, SiO_2 1.087-0.362. The amount of organic matter in solution (p.p.m.) in the two is: flat bogs 27.826, raised bogs, 0.4 to 12.741 and up to 20.86. On the assumption that the higher content of mineral matter in flat bogs than in raised bogs is more favorable to plant growth or that the low content in raised bogs is a limiting factor in plant growth the data given support the characterization of the flat bogs as eutrophic and the raised bogs as oligotrophic. The composition of the soil atmosphere and the gases dissolved in bog water as given by Bulow (*op. cit.* p. 42) do not support this view. The following is a free translation and slight condensation of von Bulow's account of these: "The amount of air in bogs is conditioned by the amount of space in the peat and the height of the water table. Its composition is influenced by the consumption of oxygen and the evolution of carbon dioxide. The air present in peat is more (in flat bogs) or less (in raised acid bogs) poor in oxygen and rich in carbon dioxide. In climatically unfavorable regions (damp, cool raised bog regions) there is a smaller content of carbon dioxide and a higher content of oxygen. Hydrocarbon gases, especially methane, are common under conditions of decay and peat formation. Nitrogen and carbon monoxide also

occur. Hydrogen sulphide is very common, especially in certain types of flat bogs. Methane is less soluble than hydrogen sulphide and thus escapes unless held back by an impermeable layer of peat, clay or other material." The work of Rigg, Thompson, Lorah and Williams (1927) agrees with this as to the high content of carbon dioxide and low content of oxygen in bog waters, and all their work was done on flat bogs. They found methane in bogs with a pond in the center, but not in drier bogs. It is difficult to believe that the high content of carbon dioxide and low content of oxygen occurring in flat bogs is favorable to plant growth (*cf.* Clements 1921). Neither can methane be considered especially favorable. It seems that calling a bog "eutrophic" because its soil water has a high content of mineral and organic matter, while neglecting the unfavorable gas conditions, is an unsatisfactory characterization. Any classification on the basis of nutrition should certainly take into account all the factors affecting plant growth. Moreover, there is no distinction between flat bogs and raised bogs based on the growth of plants in them. It is true that most raised bogs seen by the writer in North America tend more to the shrub stage than to the herb stage, but this is a difference in the stage of succession and not in the vigor of the growth of plants. Since, therefore, the only way to recognize flat bogs and raised bogs in the field is by the form of the surface, and the proposed classification as eutrophic and oligotrophic does not take into account the entire complex of factors in plant growth, it would seem that the classification of sphagnum bogs in North America as flat and raised is the most useful one. Also, the writer has not seen data for North American bogs comparing the amount of mineral and organic matter in the two types. It seems quite possible that the same conditions found in Europe might be found in North America, but we do not know that this is so and should not use it as a basis of classification until we have data, even if we did not reject it on the above grounds. It is worthy of note that in the classification of peat deposits in the United States by Dachnowski-Stokes (1933) he puts the flat sphagnum bogs of the State of Washington in his oligotrophic group. He mentions specifically four bogs of this State (Cottage Lake, Evans Creek, Ronald and Esperance), all of which are well known to the writer and are distinctly flat bogs formed in comparatively deep depressions and showing the lake type of development.

EVIDENCE ON THE COURSE OF BOG DEVELOPMENT

The data used in arriving at an understanding of the course of development of a sphagnum bog are secured by (1) a study of the plant community occupying it, (2) a study of the plant community, or communities, occupying the area immediately surrounding it, (3) the observation of any plant communities in the same region that may represent early stages in bog development, (4) making enough borings in the bog to determine the composition and thickness of the various layers of peat in it, (5) a determination (also by boring) of the nature of the inorganic material on which the bog rests, (6) a determination of the nature of the soil (or soils) of the area immediately surrounding the bog, (7) a consideration of the topography and historical geology of the region, and (8) a study of the physiological and ecological conditions under which plants grow in the bog and on the neighboring hard soil. When a trained observer looks at a sphagnum bog he sees it as something that is dynamic, not static. He thinks of it as a stage in development. He has in mind certain general principles of plant succession in bogs and looks for the evidences that will indicate whether the bog that he is examining fits readily into the general pattern or will indicate that the succession in this bog has some special features.

The plant communities occupying sphagnum bogs at their various stages of development are so strikingly different from other plant communities that this phase of bog investigation has had the attention of many workers. Such studies have been made, for example, by Nichols (1915, 1918), Transeau (1903), Lewis and Dowding (1926), Lewis, Dowding, and Moss (1928), Rigg (1914, 1916a, 1919, 1925, 1937), and Osvald (1933). Most of these papers deal with evidences of plant succession in the development of sphagnum bogs, giving attention also to the flora of the areas immediately surrounding the bogs, and recording the roles played by various pioneer species in the encroachment of bogs on other plant communities or on the open water of ponds and lakes. Lists of bog plants with some discussion of their special characteristics are given earlier in this paper. Early stages in bog succession have been discussed by the writer (Rigg 1919, 1940).

The making of borings in bogs is a laborious task. Two types of borers are available for this work—the Hillier borer, manufactured in Sweden, and the Davis borer, manufactured in the United

States. A number of workers have also devised their own borers. Bog profiles showing various courses of development have been published by Bulow (1929) who has also given an "ideal" profile. Auer (1930) gives profiles of bogs of southeastern Canada. Dachnowski-Stokes (1933) has published bog profiles and has given data on the layers of peat in many bogs of various parts of the United States. Rigg and Richardson (1934, 1938) have published profiles of Pacific coast bogs, and Rigg (1937) has given a profile of an Alaskan bog and (1940) of some bogs of eastern and north central United States. Much information about the strata of peat in bogs has been obtained by workers who were primarily interested in pollen studies (Cain 1939; Sears 1932, 1934, 1935; and several others). In many of the studies of fossil pollen in bogs the graphic presentation of the pollen data is accompanied by a profile of the peat strata at the point at which the pollen was obtained (Hansen 1939, 1939a, 1940; and others).

Much progress in the understanding of bog development has been made by means of ecological and physiological studies, especially those that report definite determinations of physico-chemical conditions produced by living sphagnum and by sphagnum peat. These have been discussed under an earlier heading. The properties of other kinds of peat have also been stated. In addition to these the relative temperature in bog soils and neighboring hard soil has been investigated. The writer (Rigg 1916), using United States Weather Bureau data on Wisconsin bogs published by Cox (1910), found that the temperatures in sphagnum during the growing season are lower than those on the neighboring hard land, and that temperatures in sphagnum moss are lower than in scalped peat. Cox (*op. cit.*, p. 119) says ". . . frost remains in the soil of an unflooded bog until comparatively late in the season, and there have been found instances of frost in the soil in marshes as late as July 4." On April 23 and 24, 1935 the writer (Rigg 1940) found three inches of frost that had to be broken with an iron bar in making borings in two bogs near Harrington, Maine. He also (Rigg 1916) computed from data by Cox (1910) that the difference between the soil temperatures and the air temperatures in Wisconsin bogs were greater than on neighboring sandy loam. He also found that the relative humidity of the air over the bog was greater than that of the air over the neighboring hard soil.

THE COURSE OF DEVELOPMENT IN FLAT SPHAGNUM BOGS

Flat sphagnum bogs are formed in depressions, usually on lakes or ponds, but occasionally on swamps. It is impossible to distinguish absolutely in some cases whether the origin was on a swamp or on a very shallow pond, since swamps have water in them at least during a portion of the year. However, when there are distinct layers of mud and sedimentary peat we may be safe in assuming that the bog originated on a lake or pond, and if these are absent and the bog is shallow a swamp origin is indicated. Bogs of these two kinds have been discussed by Rigg and Richardson (1938). Bogs of pond or lake origin vary from less than ten feet, as illustrated by Helmetta bog in New Jersey (Rigg 1940), to forty feet as illustrated by Paulsbo bog in the State of Washington (Rigg and Richardson 1938). Some sphagnum bogs of swamp origin have a depth of as much as ten feet while others are as shallow as four feet (Rigg and Richardson 1938).

Bogs are composed of stratified peat. In those formed on lakes or ponds a common order is lake mud, sedimentary peat, sedge peat, sphagnum peat. Bogs formed on lakes in glaciated regions commonly have a layer of blue clay underlying the lake mud. It seems probable that this blue clay is outwash material from the retreating glacier, ground to extreme fineness by the movement of the ice. This clay is relatively impermeable to water and prevents seepage from the lake into the glacial till, thus insuring permanency for the lake. The layer of lake mud is next washed into the lake from the surrounding slopes and settles into the lake from the air, though undoubtedly to a lesser extent. A layer of mud several feet in thickness may thus accumulate. The fact that the layer of mud is irregular in thickness supports the view expressed above that inwash is a larger factor than settling from the air. Naturally some organic matter will be washed in with the mineral particles which form the larger portion of the soil around the lakes. Some organisms will flourish in the lakes even when the water is still very cold because of the inflow from the melting glaciers. As the glaciers retreat and the lakes cease to receive ice water their waters become warmer and more organisms flourish in them. The remains of the organisms settle to the bottom and form sedimentary peat. As long as the water of the lake is muddy, however, some mineral matter will be deposited. From the above it is evident that the

transition from lake mud to sedimentary peat in lakes formed in a region of retreating glaciers is natural, but may be gradual and the line between them may not be sharp.

Sedge peat in bogs of lake origin has probably in most cases originated as a floating mat from the bottom of which disintegrating plant remains dropped into the water and which eventually became so heavy that it sank to the bottom. Undoubtedly it was in many cases overrun by sphagnum before the sinking occurred and was gradually forced down by the increasing weight. It is not conceivable that sedges grew on the bottom of a lake many feet in depth, but we must always keep in mind the possibility of changes in the level of the lake due to local changes which may have increased or decreased either the outflow or the inflow of the lake. A possible hypothesis for the formation of sedge peat *in situ* would be such changes in the level of the lake. Evidences for such changes are not now apparent in the sphagnum bogs examined by the writer, but plenty of cases are found where the level of lakes has been raised by beaver dams and by slides and also where the level has been lowered by the water cutting into soft soil. The surface layer of sphagnum is commonly raw, and directly under it in many cases is a layer of disintegrated sphagnum (Rigg and Richardson 1938). The line between the two is commonly sharp. There is usually a layer of living sphagnum at the surface of flat sphagnum bogs. This may form a continuous layer or may be present only in patches. The sphagnum layer commonly originates as a mat, but it is not itself the pioneer in mat formation (Rigg 1925). The pioneers in the formation of a sphagnum mat may be either shrubs or herbs. *Chamaedaphne*, *Kalmia* and *Ledum* are the commonest shrubs which grow forward into the water, and their stems curve upward so that their tips and leafy portions are in air. They thus form a support on which *Sphagnum* grows. *Sphagnum* is commonly preceded or accompanied in this type of mat formation by *Carex* and *Drosera* with *Rhynchospora* following close behind. Two herbs which commonly function as pioneers in mat formation are *Menyanthes trifoliata* and *Potentilla palustre*. Their long slender stems grow in the water forming a support on which *Carex*, *Drosera*, *Rhynchospora* and *Sphagnum* follow much as they do on the shrub pioneers. Some bogs which began as a circle of sphagnum around a pond or small lake still have open water in the center,

while others have gone on to the stage where the open water is entirely obliterated (Rigg and Richardson 1938). Large pockets of water under the sphagnum layer are common in bogs formed on lakes (Rigg 1940). Sphagnum bogs often originate on a portion of the margin of larger lakes without surrounding them. Small sphagnum bogs sometimes originate on logs which have lain for a long time in the same position in a pond or lake. *Carex* and *Drosera* commonly appear first in such cases in the Puget Sound region, and are followed by *Kalmia* and *Sphagnum*. All stages in this up to the establishment of the typical bog community are seen. An excellent example of sphagnum bog succession around a pond is seen at Spruce Hole near Durham, New Hampshire (Hodgdon 1932). Large sphagnum bogs or numerous small patches of bog often float on lakes. An example of the former is Cranberry Island on Buckeye Lake in Ohio (Detmers 1912 and Dachnowski 1911), and an example of the latter is seen on Fish Lake in Glacier National Park.

Flat sphagnum bogs commonly have a "marginal ditch" or "mote" around them. In the Puget Sound region this usually contains water a foot or more in depth during the mild rainy winters which are characteristic of the region, and may be dry enough to walk on during the summer when there is little rain. The soil in the marginal ditch is mostly muck which originates from the continued inwash of mineral soil and the growth and disintegration of plants in this soil and the water which covers it. While the sphagnum area of such a bog has a typical flora of bog herbs or shrubs or a mixture of the two, the marginal ditch is occupied by swamp herbs or shrubs, the latter frequently forming a dense thicket.

Where sphagnum bogs are formed on very shallow ponds, *Sphagnum* grows in the water without the support of other plants. This type of development is seen in Buckingham bog in New Jersey (Rigg 1940). Early stages in which *Sphagnum* is just beginning to grow are sometimes found. An example of this is seen in the heath pond at Onga Hat, New Jersey (Rigg 1940).

Examples of sphagnum bogs that have originated on swamps are seen in the State of Washington at Seabeck, Echo Lake and Forks. Early stages of this type of development are common (Rigg 1919). The bottom layer in such bogs is usually sedge or muck. Where a sphagnum bog borders on a swamp the bog is usually encroaching on the swamp. Examples of sphagnum bogs encroaching on sedge or hardhack swamps are common in the State of Washington.

THE COURSE OF DEVELOPMENT IN RAISED SPHAGNUM BOGS

It has already been noted that raised sphagnum bogs differ from flat sphagnum bogs in both origin and course of development. Raised sphagnum bogs are formed in saucer- or trough-shaped depressions or on undulating flat surfaces or on slopes (Transeau 1903, 1906; Soper 1919; Nichols 1915, 1918; Rigg 1940). The essential condition is that there be a water supply on the surface which can be drawn upward through the accumulating mass of plant remains by capillarity and imbibition as it is used by growing plants or is lost by evaporation. This water is frequently of subterranean origin (Nichols 1915, 1918; Rigg 1940), but in some cases there is no evidence of this and the water seems to originate from the abundant rain and snow on neighboring hills or mountains.

The number of layers of peat found in raised bogs is commonly smaller than in flat bogs and their development is thus simpler. Many raised bogs show only three strata of peat and some show only two. Where the depression is quite shallow the three layers may be sedge, wood and sphagnum (Rigg 1937). Where it is a little deeper and there is no drainage from the level of the bottom the three layers may be sedimentary peat, sedge peat and sphagnum (Rigg 1940). The convexity of raised sphagnum bogs is most frequently due entirely to the growth of *Sphagnum*, the other layers being either flat or concave. In some raised bogs, however, the borings show clearly that the growth of sedges and similar plants and the accumulation of their remains has caused the convexity (Rigg 1940).

Raised sphagnum bogs commonly occur near the sea, and oceanic climates are certainly more favorable to them than the continental climates (Nichols 1915, 1918). Iron Spring bog in Minnesota is the only exception known to the writer. Raised sphagnum bogs in North America are most common in northerly regions and do not extend as far south as flat bogs (Rigg 1940). Large raised sphagnum bogs occurring on flat or undulating surfaces may show a rise of one to five feet at the margin and have a gentle slope over the rest of the surface (Rigg 1940). Very large raised sphagnum bogs may look flat because of their great area and the comparatively small differences in elevation between the margin and the center. Many of the raised sphagnum bogs of North America are in the shrub stage, but also have many herbs. Since many flat bogs are

also in the shrub stage we cannot say that the shrub stage at the present time is a distinguishing character of raised sphagnum bogs. When we attempt to make broad generalizations about the origin and development of raised sphagnum bogs we are confronted with so many exceptions that it is necessary in the present state of our knowledge to study each case carefully and determine all the factors, seeking always to find out which one or ones are determining factors in each case. Some generalizations have been made on the basis of bogs of the Atlantic coast only, which must be modified in the light of knowledge of the bogs of the Pacific coast and the northern portion of the interior. Generalizations when once recorded in the literature are likely to find their way into texts and reference books and are then difficult to correct. The generalizations by Nichols (1918) that the origin of water for the development of raised sphagnum bogs is meteoric rather than telluric, and that *Sphagnum* plays a major role in the development of raised bogs, have to be modified in the light of later knowledge extending over a wider area (Rigg 1940). The generalizations with reference to flat bogs have shown less error than those in regard to raised bogs, but even in this type all conditions in each bog should be fully determined and carefully considered. It is more than possible that the above criticisms may be successfully applied to some of the generalizations in this paper, but the writer can only plead that he has tried to be cautious.

THE GENERAL COURSE OF BOG DEVELOPMENT

There are some generalizations about the course of development of sphagnum bogs in North America that either apply clearly to both flat bogs and raised bogs or, if they are especially clear in one type, they are also more or less evident in the other.

Tundra, muskeg, and sphagnum bog are successive stages in bog development. In Alaska it seems clear to the writer that the first of these merges into the second and the second into the third. Either of the first two may be climax so far as field evidence at the present time is an indication, but at some places in Alaska there is clear evidence that they are successive stages in development. This has been discussed by Rigg (1937). The term "muskeg" has been used by Lewis and Dowding (1926) and others as a synonym for bog, and popular use in Alaska does not distinguish between the

two terms. It is the belief of the writer, however, that the recognition of the successive relation of tundra, muskeg and sphagnum bog leads to clearness of thinking about the plant successions involved in them.

There are a good many areas, especially in the Puget Sound region, which have a swamp flora (sedge or hardhack) but show stratification of the peat beneath. The general course of development in these is the same as that of sphagnum bogs except that they do not have the sphagnum layer at the surface. Perhaps it is mere chance that *Sphagnum* never got started on them, since they occur in the immediate vicinity of sphagnum bogs showing a similar course of development. Where a sphagnum bog borders a swamp which is characterized by sedges or hardhack the bog is encroaching on the swamp, but cases where sphagnum is entirely lacking are often seen.

Mosses other than *Sphagnum* often take part in the development of sphagnum bogs. Cooper (1912) has worked out the part played by *Sphagnum* and other mosses in the development of plant communities, including bogs, on Isle Royale. Among the mosses discussed are *Polytrichum*, *Calliergon*, *Camptothecium*, *Hylocomium* and *Hypnum*. Bastin and Davis (1909) and Auer (1927) have also given data on various mosses in bogs. A layer of *Hypnum* peat occurs in some sphagnum bogs of the Puget Sound region (Rigg and Richardson 1938). In one bog this layer is six feet in thickness. In some bog profiles a continuous layer of sedge peat is seen directly over the *Hypnum* layer and in others it is covered directly in one portion by wood peat and in another portion by reed peat.

Many sphagnum bogs are characterized by hummocks on the surface. In some cases these have grown upward on a comparatively level surface, while in others these are meandering, morass-like depressions between the hummocks. Dokturowski (1931) has described these in European bogs and the writer has often observed them in North American bogs.

Some sphagnum bogs occur at rather high elevations. These are not usually so extensive as those at lower levels and some of them are very small. Those of Mazama Dome in the State of Washington (Rigg 1922a) are at an elevation of 6500 feet and are mere ridges two or three feet thick and a few feet in height and 20 to 30

feet long forming the lower side of small ponds, the other sides of which are rock. Small shallow sphagnum bogs occur in Berkeley Park on Mount Rainier at an elevation of 5000 feet (Rigg 1935). Other sphagnum bogs of larger area with a thin layer of sphagnum occur at other points in the Cascade Mountains.

Sphagnum bogs with rivers flowing through them are fairly common. This phenomenon is seen near the Atlantic coast and in the northern portion of the interior but does not occur, so far as the writer knows, on the Pacific coast (Rigg 1940). The most striking example of this seen by the writer is Great Heath near Harrington, Maine, through which the Columbia river (locally called Pleasant River) flows very slowly in a meandering course and receives some creeks as tributaries. The considerable areas of standing ("flow-age") water in this bog at the time it was visited (April 23, 1935) indicates that the surface of the portion of this huge bog that was seen is approximately level though it rises rather abruptly about five feet at the margin. It does not appear that this river which moves so slowly through the enormous bog area but forms swiftly in a narrow channel near its mouth really provides much active drainage in the bog. The writer has no information as to the relation of the rivers to the other bogs reported as having them (Auer 1927; Cooper 1938). Small creeks flow slowly through some Puget Sound sphagnum bogs, but no large streams have been seen. It seems to the writer, so far as the facts are known, that sphagnum bogs, even those with rivers flowing through them, are poorly drained habitats.

A number of sphagnum bogs on the Atlantic coast are now being eroded by the sea, but this phenomenon has not been seen on the Pacific coast. A number of examples are seen on the coasts of Maine and New Brunswick. (Dachnowski-Stokes 1930a; Auer 1933; Rigg 1940.) It is not a steady, day by day removal but takes place mostly during storms at high tide. Two factors, a recent subsidence of the coast and the removal of barrier beaches by shifting marine currents have undoubtedly operated in exposing these peat deposits to the action of the sea (Dachnowski-Stokes 1930).

Sphagnum sometimes covers a plant community in which a dense growth of shrubs has been dominant. In Carncross bog on Lulu Island, B. C., the erect shrubs *in situ* are readily exposed by digging a few feet. This is a delta bog, the flat island on which it has

developed being between the two arms of the Fraser River near its mouth. Early stages of the growth of sphagnum on the ground in a hardhack swamp are seen in the Puget Sound region (Rigg 1919), though the topographic conditions seen do not indicate that the sphagnum, even if the areas had not been disturbed by man's activities, would ever cover the shrubs completely.

The climax stage of a sphagnum bog is commonly a forest. Undoubtedly there are exceptions to this, but the experience of the writer in many years of studying bogs of the Pacific Coast of the United States, Canada and Alaska and what study he has made of bogs in other portions of the United States as well as his study of bog literature leads him to believe that the generalization that sphagnum bogs, as they mature, tend toward a forest stage is sufficiently valid to be useful. MacMillan (1896) has stated this generalization for the bogs of the Minnesota River valley. Transeau (1903) has made it a generalization for bog societies and (1905) has made diagrams showing the stages in development from aquatic plants through sedge stages and bog shrub stages to coniferous forest. Bastin and Davis (1909) and Nichols (1915) have described tree stages in bogs which accord with the generalization. The writer (Rigg 1922) has described a climax bog forest of lodge pole pine (*P. contorta*) with an undergrowth of salal (*Gaultheria shallon*) and Labrador tea (*Ledum groenlandicum*) on a sphagnum bog near Victoria, B. C. The coming of the climax here has probably been hastened somewhat by artificial drainage. Early stages in the invasion of sphagnum bogs by trees are readily found in almost any bog region.

The forest trees that invade sphagnum bogs in their more or less mature stages of development and become the dominant vegetation in very late stages are mostly conifers. Among the conifers that commonly take part in this development are spruces, tamaracks, cedars, pines and hemlocks. Some deciduous trees also invade sphagnum bogs. The most common of these are birches (Bastin and Davis 1909; Rigg 1922*b*). Alders, ashes and red maple have also been reported (Bastin and Davis 1909).

Trees growing in sphagnum are usually stunted. The writer (Rigg 1918) has made a study of the diameter, height, and number of annual rings in trees in sphagnum bogs and on the neighboring hard soil in the Puget Sound region. The western hemlock

(*Tsuga heterophylla*) is the earliest invader of these bogs and comes the nearest to attaining its normal growth in them, while the Douglas fir (*Pseudotsuga taxifolia*) is the last invader and is the most stunted by bog conditions. Between these two extremes are lodge pole pine (*P. contorta*), western white pine (*P. monticola*), and western red cedar (*Thuja plicata*). The two species of pines mentioned are common invaders of sphagnum bogs in the Puget Sound region, and sometimes the one and sometimes the other forms an almost pure stand in the bogs. Occasional places have been found in sphagnum bogs in which lodge pole pine shows rapid growth, but on investigation these have been found to be near ponds, or swampy areas where the usual bog conditions do not prevail. Stunted trees in bogs have been observed by the writer in many sphagnum bogs of the Pacific coast and by other workers elsewhere.

The roots of forest trees growing in sphagnum show special forms (Rigg and Harrar 1931). Many of them are not round in cross section but have either an I-beam or T-girder form, thus showing greater growth on either the top or bottom or both than on the sides. The root systems are shallow, being limited in their downward growth by the level of the water table during the growing season. They have a very wide extent laterally, and many natural root grafts occur, thus forming a yielding but strong mat in the soft substratum. No windfalls were found in sphagnum areas, though they are common on neighboring glacial till. Nature has here done a remarkable job of engineering in providing effective anchorage in a yielding substratum. It is believed that the I-beam and T-girder forms of these roots are to be interpreted as growth in the direction of the strain put upon them as the trees sway in the wind. The specialized forms and growth habits here described are not confined wholly to sphagnum, but are found to a less extent in swampy habitats.

Many "bog theories" have been proposed which attempt to account for the presence of the distinctly characteristic bog flora and the exclusion of the ordinary plants of the region. These have been stated and discussed, and literature relating to them cited by the writer (Rigg 1916a). The 35 theories presented are grouped under four heads: (1) Why are plants characteristic of sphagnum bogs mainly xerophytic? (2) How are plants other than bog xerophytes

inhibited from sphagnum bogs? (3) What are the possible sources of the toxic substance or substances in bog water? (4) How do the toxic substances in bog water act on plants? It is now evident that the most hopeful approach to the study of bog problems is the quantitative determinations of the factors involved rather than attempts to justify sweeping generalizations expressed in the form of theories without adequate data to support them.

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